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(54) **Title:** DNA-ENCODED CHEMICAL LIBRARIES

(57) **Abstract:** A method of preparing a DNA-encoded chemical library comprising members which comprise a chemical moiety, a linking moiety and a DNA moiety, comprising for each member the steps of: (I) coupling a initial building block to a initial coding single strand oligomer via a linking moiety to form a initial conjugate, the initial coding single strand oligomer comprising a first primer region, an initial coding region and a first annealing region; (II) optionally coupling a middle building block and middle coding single strand oligomer to said initial conjugate to form a middle conjugate, the coupling take place in either order, wherein: (a) the middle building block is coupled to the residue of the initial building block; and (b) the middle coding single strand oligomer comprises a middle coding region and second annealing region, and is coupled by: (i) annealing a complementary single strand oligomer which comprises a chemical modifier, a complementary first annealing region, a complementary middle coding region and a complementary second annealing region by interaction between the first annealing region and the complementary first annealing region; (ii) treating the conjugate formed with a DNA polymerase to elongate the initial coding single strand oligomer to be complementary to the complementary middle coding region and the complementary second annealing region; (iii) removing the complementary single strand oligomer by denaturing the DNA and capturing the complementary single strand oligomer via the chemical modifier; (III) coupling a final building block and final coding single strand oligomer to the initial or middle conjugate as appropriate to form a final conjugate, the coupling take place in either order, wherein: (a) the final building block is coupled to the residue of the initial building block, or may be additionally or alternatively be coupled to the residue of the middle building block (if present); and (b) the final coding single strand oligomer comprises a final coding region and a second primer region, and is coupled by: (i) annealing a complementary single strand oligomer which comprises a complementary first or second annealing region as appropriate, a complementary final coding region and a complementary second primer region, by interaction between the first or second annealing region and the complementary first or second annealing region, as appropriate; (ii) treating the conjugate formed with a DNA polymerase to elongate the initial coding single strand oligomer to be complementary to the complementary final coding region and the complementary second primer region, and to elongate the complementary coding strand to be complementary to the initial coding single strand oligomer.

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DNA-ENCODED CHEMICAL LIBRARIES

The present invention relates to methods of preparing DNA-encoded chemical libraries, libraries prepared by those methods, use of these libraries and particular compounds therein.

Introduction

Identification of molecules that bind specifically to target proteins of interest is a formidable challenge. Technologies that facilitate the isolation of binding molecules have profound implications for pharmaceutical research because most drug development programs rely on the ability to isolate small organic compounds that bind to a given protein. With an aging population and an increased understanding of the mechanisms of disease at a molecular level, biomedical scientists are facing the demand for more and better drugs. Additionally, elucidation of the biological function of proteins will, in many cases, require access to specific ligands (an approach that is often termed 'Chemical Genetics' (Strausberg, R.L. and Schreiber, S.L., Science **300** (2003), 294–295). Techniques for the general, fast, inexpensive isolation of small, organic, binding molecules are lacking at present.

Traditional high-throughput screening (HTS) procedures require the storage and handling of hundreds of thousands of chemical compounds and, typically, they rely on a biochemical assay (Keseru, G.M. and Makara, G.M., Drug Discov. Today **11** (2006), 741–748). Alternatives to HTS that have been introduced recently include dynamic combinatorial chemistry (Lehn, J.M. and Eliseev, A.V., Science **291** (2001), 2331–2332; Otto, S., et al.,Drug Discov. Today **7** (2002), 117–125), small-molecule microarrays (Uttamchandani, M., et al., Curr. Opin. Chem. Biol. **9** (2005), 4–13), fragment-based lead discovery (Carr, R.A., et al., Drug Discov. Today **10** (2005), 987–992; Erlanson, D.A., Curr. Opin. Biotechnol. **17** (2006), 643–652) and DNA-encoded chemical libraries (Dumelin, C.E., et al., QSAR Comb. Sci. **25** (2006), 1081–1087; Scheuermann, J., et al., J. Biotechnol. **126** (2006), 568–581). DNA-encoded chemical libraries are collections of small organic molecules that are conjugated covalently to DNA tags that serve as identification bar codes.

In DNA-encoded chemical library, the organic molecule, or putative binding moiety, is linked physically to a DNA tag that carries the identity code of the molecule it is attached to. The selection procedure for DNA-encoded chemical libraries comprises incubating the library of encoded molecules with the target protein of choice and, after affinity capture, non-binding library members are separated from binding library members by, for example, coupling the target protein to a solid support and removing the supernatant (that contains the non-binders).

The stringency of selection is controlled using suitable washing conditions. After elution of the DNA-tagged binders, the enriched DNA moieties are amplified by polymerase chain reaction (PCR), which allows very low amounts of template DNA to be detected. Subsequent decoding of the enriched DNA uses either DNA sequencing or hybridization to oligonucleotide microarrays, depending on the architecture of the library and its size. In some libraries, the DNA tags not only serve as identification bar codes, but can also guide the synthesis of the displayed molecule.

It is probable that the concept for the construction of a DNA-encoded chemical library was proposed first by Sydney Brenner and Richard Lerner in 1992 (Brenner, S. and Lerner, R.A.. *Proc. Natl. Acad. Sci. U. S. A.* **89** (1992), 5381–5383; US 5,573,905). The authors postulated the alternating stepwise synthesis of a polymer (e.g. a peptide) and an oligonucleotide sequence (serving as an identification bar code) on a common linker (e.g. a bead) in split and pool cycles. After affinity capture on a target protein, the population of DNA tags of the selected library members would be amplified by PCR and, in theory, utilized for enrichment of the bound molecules by serial hybridization steps to a subset of the library. In principle, the affinity-capture procedure could be repeated, possibly resulting in a further enrichment of the active library members. Finally, the structures of the chemical entities would be decoded by cloning and sequencing the PCR products. The feasibility of the orthogonal, solid-phase synthesis of peptides and oligonucleotides was demonstrated by attaching a test peptide (the pentapeptide leucine enkephalin) and an encoding DNA tag onto controlled-pore glass beads (Nielsen, J., *et al.*, *J. Am. Chem. Soc.* **115** (1993)). The peptide bound to a specific antibody and the corresponding DNA coding tag was amplified by PCR. To date, the only implementation of the technology at a library level has been published by scientists at Affymax, who have constructed a collection of ~10^6 heptapeptide sequences (based on seven amino acids) and their corresponding coding oligonucleotide tags on beads. The library was incubated with a fluorescently labelled anti-peptide antibody, and binders were selected successfully by fluorescent-assisted cell sorting (Needels *et al.*, M.C., *Proc. Natl. Acad. Sci. U. S. A.* **90** (1993), 10700–10704). In the original paper, Brenner and Lerner suggested that the alternate synthesis of chemical compounds and oligonucleotides could also be implemented in the absence of beads. This approach has been followed by the companies NuEvolution (US 2006-0246450; WO 02/103008) and Praecis (WO 2005/058479) by using enzyme-catalyzed ligation of coding DNA blocks rather than chemical synthesis of oligonucleotides.

Other methods of synthesizing DNA-encoded chemical libraries involve DNA-templated organic synthesis (Gartner, Z.J., *et al.*, *Science* **305** (2004), 1601–1605) and encoded self-assembly

chemical libraries (Melkko, S., *et al.*, *Nat. Biotechnol.* **22** (2004), 568–574; Sprinz, K.I., *et al.*, *Bioorg. Med. Chem. Lett.* **15** (2005), pp. 3908–3911.)

General Description

The present inventors have developed a method of preparing DNA-encoded chemical libraries in which the initial coding of each building block of the chemical moiety is carried out by a single stranded oligomer, and where the double stranded DNA moiety in the final library members is completed using a DNA polymerase.

A first aspect of the present invention provides a method of preparing a DNA-encoded chemical library comprising members which comprise a chemical moiety, a linking moiety and a DNA moiety, comprising for each member the steps of:

(I) coupling a initial building block to a initial coding single strand oligomer via a linking moiety to form a initial conjugate, the initial coding single strand oligomer comprising a first primer region, an initial coding region and a first annealing region;

(II) optionally coupling a middle building block and middle coding single strand oligomer to said initial conjugate to form a middle conjugate, the coupling take place in either order, wherein:

(a) the middle building block is coupled to the residue of the initial building block; and

(b) the middle coding single strand oligomer comprises a middle coding region and second annealing region, and is coupled by:

(i) annealing a complementary single strand oligomer which comprises a chemical modifier, a complementary first annealing region, a complementary middle coding region and a complementary second annealing region by interaction between the first annealing region and the complementary first annealing region;

(ii) treating the conjugate formed with a DNA polymerase to elongate the initial coding single strand oligomer to be complementary to the complementary middle coding region and the complementary second annealing region;

(iii) removing the complementary single strand oligomer by denaturing the DNA and capturing the complementary single strand oligomer via the chemical modifier;

(III) coupling a final building block and final coding single strand oligomer to the initial or middle conjugate as appropriate to form a final conjugate, the coupling take place in either order, wherein:

(a) the final building block is coupled to the residue of the initial building block, or may be additionally or alternatively be coupled to the residue of the middle building block (if present); and

(b) the final coding single strand oligomer comprises a final coding region and a second primer region, and is coupled by:

(i) annealing a complementary single strand oligomer which comprises a complementary first or second annealing region as appropriate, a complementary final coding region and a complementary second primer region, by interaction between the first or second annealing region and the complementary first or second annealing region, as appropriate;

(ii) treating the conjugate formed with a DNA polymerase to elongate the initial coding single strand oligomer to be complementary to the complementary final coding region and the complementary second primer region, and to elongate the complementary coding strand to be complementary to the initial coding single strand oligomer.

A second aspect of the present invention provides a method of preparing a DNA-encoded chemical library comprising a chemical moiety, a linking moiety and a DNA moiety, comprising for each member the steps of:

(I) coupling a initial building block to a initial coding single strand oligomer via a linking moiety to form a initial conjugate, the initial coding single strand oligomer comprising a primer region, an initial coding region and an annealing region;

(II) coupling a middle building block and middle coding single strand oligomer to said initial conjugate to form a middle conjugate, the coupling take place in either order, wherein:

(a) the middle building block is coupled to the residue of the initial building block; and

(b) the middle coding single strand oligomer comprises a middle coding region and a restriction region, and is coupled by:

(i) annealing a complementary single strand oligomer which comprises a complementary annealing region, a complementary middle coding region, a complementary restriction region and a chemical modifier, by interaction between the annealing region and the complementary annealing region;

(ii) treating the conjugate formed with a DNA polymerase to elongate the initial coding single strand oligomer to be complementary to the complementary middle coding region and the complementary restriction region and to elongate the complementary single strand oligomer to be complementary to the initial primer region and initial coding region; and

(iii) treating the conjugate with a non-palindromic restriction enzyme which acts on the restriction region to leave a middle conjugate, and capturing the restriction product via the chemical modifier;

(III) coupling a final building block and final coding single strand oligomer to the middle conjugate as appropriate to form a final conjugate, the coupling take place in either order, wherein:

(a) the final building block is coupled to the residue of the initial building block and/or to the residue of the middle building block; and

(b) the final coding single strand oligomer comprising a final coding region and a second primer region, and is coupled by ligating a terminal double-stranded portion comprising a ligation region that matches the remainder of the restriction region site of the middle conjugate, and a final coding region and second primer region, as well as the complementary regions in the complementary strand.

In both the first and second aspects of the invention, it is preferred that the individual method steps are carried out at about the same time for each library member. In particular, it is preferred steps (I), (II) if present, and (III) follow a split and pool methodology. This well-known technique in combinatorial chemisty involves carrying out step (I) in an appropriate number of vessels for the number of building blocks, pooling the resulting initial conjugates and then splitting the mixture so obtained into the appropriate number of vessels for the number of building blocks in the subsequent step. This method can be repeated a third time if the method involves three groups of building blocks.

A third aspect of the present invention provides a method of preparing a DNA-encoded chemical library comprising members which comprise a chemical moiety, a linking moiety and a DNA moiety, comprising for each member the steps of:

(I) coupling a initial building block to a initial coding single strand oligomer via a linking moiety to form a initial conjugate, the initial coding single strand oligomer comprising a first primer region, an initial coding region and a first annealing region;

(II) coupling a middle building block and middle coding single strand oligomer to said initial conjugate to form a middle conjugate, the coupling take place in either order, wherein:

(a) the middle building block is coupled to the residue of the initial building block; and

(b) the middle coding single strand oligomer comprises a middle coding region and a restriction region, and is coupled by:

(i) annealing a complementary single strand oligomer which comprises a complementary first annealing region, a complementary middle coding region and a complementary restriction region by interaction between the first annealing region and the complementary first annealing region;

(ii) treating the conjugate formed with a DNA polymerase to elongate the initial coding single strand oligomer to be complementary to the complementary middle coding region and the complementary restriction region;

(iii) treating the conjugate with a restriction enzyme which acts on the restriction region to

leave a middle conjugate, and removing the restriction product not containing the coding regions with a chromatographic method;

(III) coupling a final building block and final coding partially double stranded oligomer to the middle conjugate to form a final conjugate, the coupling take place in either order, wherein:

(a) the final building block is coupled to the residue of the initial building block, and additionally or alternatively be coupled to the residue of the middle building block; and

(b) the final coding partially double stranded oligomer comprises a final coding region, a second primer region, and a double stranded ligation region that matches the remainder of the restriction region site of the middle conjugate, and is coupled by ligation using an excess of the final coding partially double stranded oligomer.

In the third aspect of the invention, it is preferred that the individual method steps are carried out at about the same time for each library member. In particular, it is preferred steps (I), (II) and (III) follow a split and pool methodology. This well-known technique in combinatorial chemisty involves carrying out step (I) in an appropriate number of vessels for the number of building blocks, pooling the resulting initial conjugates and then splitting the mixture so obtained into the appropriate number of vessels for the number of building blocks in the subsequent steps

A fourth aspect of the present invention provides a library synthesised according to the method of either the first, second or third aspects of the invention. This aspect also comprises the particular library described below however made.

A fifth aspect of the present invention provides the use of the library of the fourth aspect in a method of screening the library for a library member which shows affinity for a biological target.

Detailed Description

Figures

The first aspect of the invention is illustrated in figures 1 and 2. Figure 1 illustrates the invention where the optional middle step doesn't take place, whereas Figure 2 illustrates the invention with the optional middle step.

The second aspect of the invention is illustrated in figure 3.

Figure 4 shows the results of the screening of a library of the invention against streptavidin; figure 4a shows the frequency of the library members before selection; figure 4b shows the frequency of the library members after selection on empty beads; figure 4c shows the frequency

of the library members after selection against Streptavidin bead, as well as the identity of high frequency library members.

Figure 5 shows the results of the screening of a library of the invention against IgG, with the identity of high frequency library members.

Figure 6 shows the results of use of an IgG binding resin according to the invention.

Figure 7 illustrates three embodiments of the first and second aspects of the invention, including gels resulting from the experiments described in Example 7.

The third aspect of the invention is illustrated in Figure 8.

Library Members

Each member of the library comprises a chemical moiety, which is made from building blocks, a DNA moiety, which includes codes for the building blocks of the member, and a linking moiety, which links the chemical moiety to the DNA moiety.

Library

A library of this invention is a collection of chemical diverse library members.

The number of different members in a library represents the complexity of a library and is defined by the number of building blocks in each member of the library, and by number of variants of each building block. The number of different members of any particular library can be determinded by multiplying the number of variants of each building block together. For example, if there are two building blocks, each of which have twenty variants, then the resulting library has 400 members. If, for example, there are three building blocks, each of which has twenty variants, then the resulting library has 8000 members.

Because the number of different members of the library will determine the amount of a particular member relative the other members in the library, there are theoretical limits to the maximum size of a library. Therefore it is useful to consider how large a library should be. This size limit is dictated by the level of sensitivity for detecting the presence of the DNA moiety after the screening process. Detection sensitivity is dictated by the threshold of binding between an acceptor molecule to be assayed and a library member.

The relative amounts of the individual members within the library can vary from about 0.2 equivalents to about 10 equivalents, where an equivalent represents the average amount of a member within the library. Preferably each member is present in the library in approximately equimolar amounts.

In a preferred embodiment, a library contains the every possible member based on the mathematical combinations for a given library.

Chemical Moiety

The chemical moiety is formed from the initial, middle (if present) and final building blocks. If there are only two building blocks, initial and final, these may be joined to one another by a chemical reaction (see below) and their residues will be linked by one or more chemical bonds.

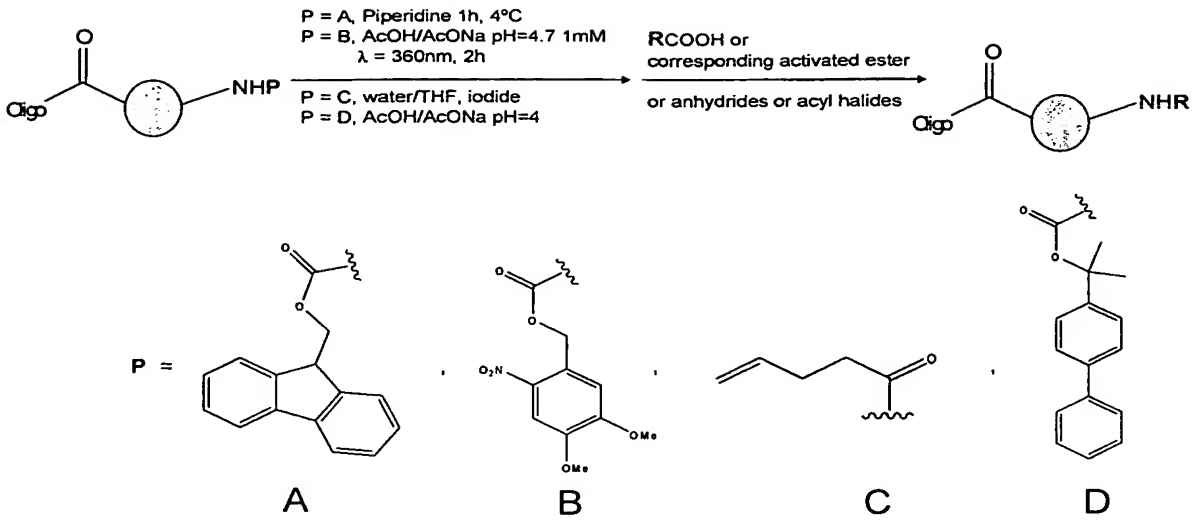
If there are three building blocks, then these can be joined in one of three ways. In a first way, the building blocks are linked in series, i.e. Initial-Middle-Final. In a second way, both middle and final building blocks are linked to the initial building block. In a third way, both middle and final building blocks are linked to the initial building block, as well as to each other other.

Accordingly, the functionality required on each building block for linking to other building blocks will vary depending on the mode of binding desired. The initial building block will also require functionality so as to bind to the linking moiety.

The reaction conditions used to link the building blocks must be compatible with the DNA moiety of the library members. Thus, the type of building blocks that are suitable for use in the present invention will be limited by suitable linking chemistry.

Illustrative building blocks and reaction schemes are presented below.

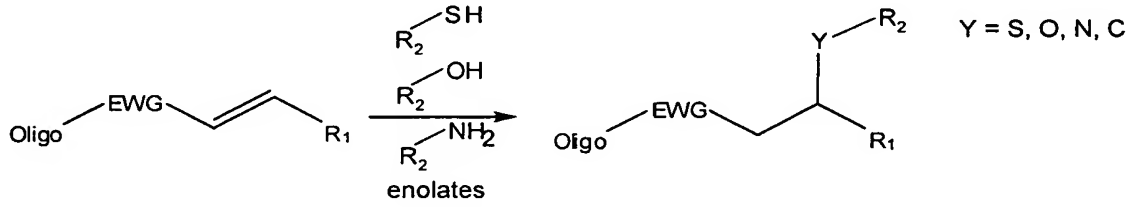
If an amino acid residue, or other amine containing moiety, (with appropriate amine protection) is the first building block, then a subsequent building block may be a carboxylic acid (or activated version thereof, e.g. ester, acid anhydride, acid halide). Appropriate conditions are shown in scheme 1, where the method is illustrated with respect to an amino acid residue:



Scheme 1

Thus, in some embodiments, if the first building block comprises a protected amine group, this is deprotected before being coupled with a carboxylic acid or activated form thereof.

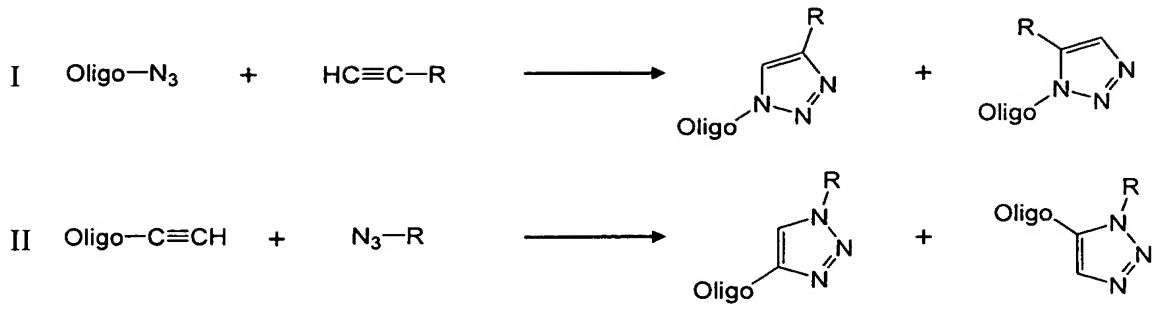
If the first building block comprises a double bond conjugated to an electron withdrawing group, then a subsequent building block being a thiol, alcohol, primary amine or enolate may be added, as shown in scheme 2:



EWG = Elctron Withdrawing Group

Scheme 2

Click chemistry may be used to join an azide containing moiety to a alkyne containing moiety, as shown in scheme 3:

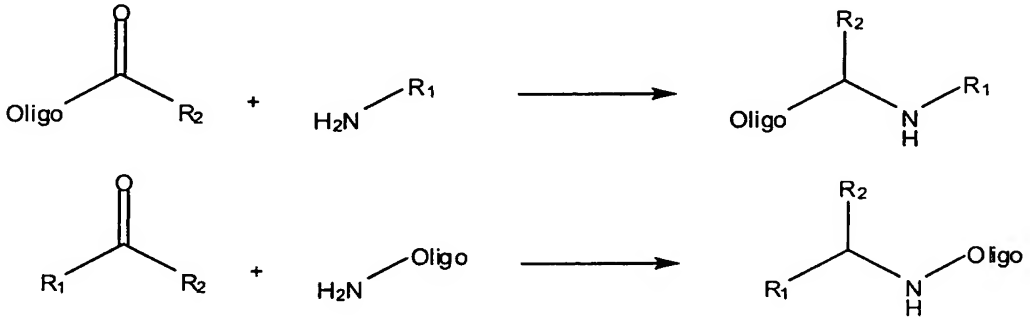


Scheme 3

Ether-bond formation could be used to join a moiety comprising a hydroxy group and a moiety comprising a leaving group, such as halogen, OTf, ONf or OTs, as shown in scheme 4:

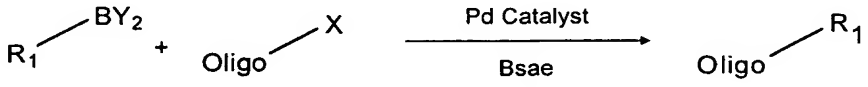
Scheme 4

Reductive amination could be used to link a moiety comprising a ketone group with a moiety comprising a primary amine group, as shown in scheme 5:



Scheme 5

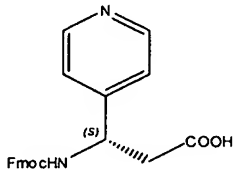
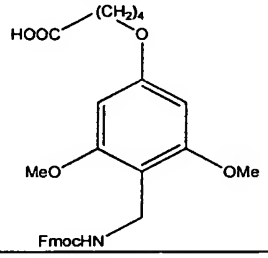
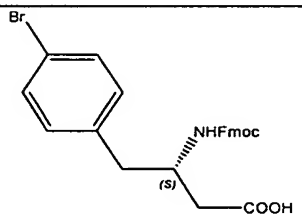
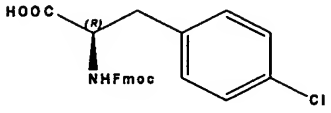
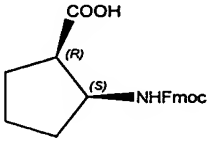
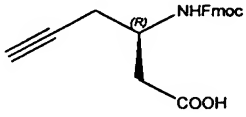
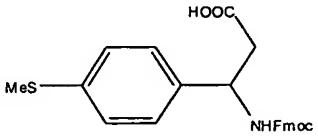
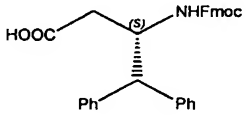
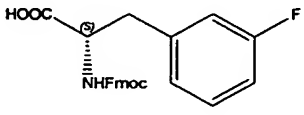
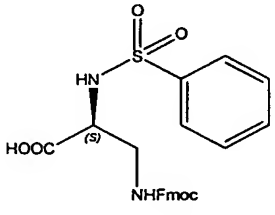
Suzuki cross-coupling may be used to link a boron containing moiety to a moiety with a leaving group, such as I, OTf, Br or Cl, as shown in scheme 6:

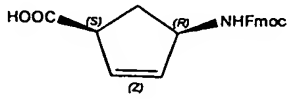
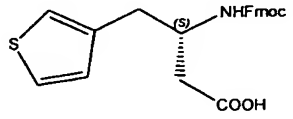
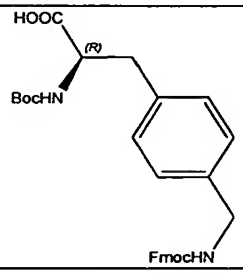
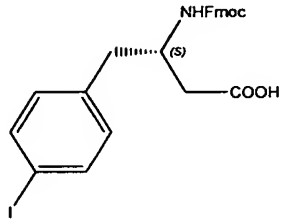
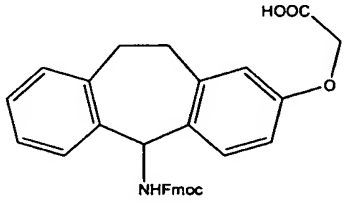
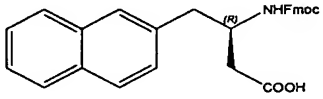
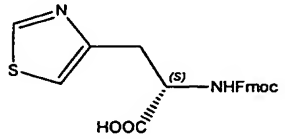
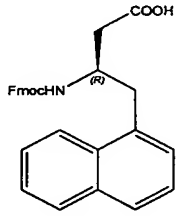
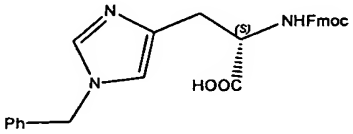
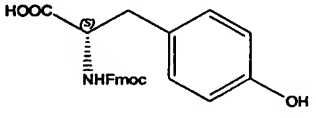


X = I, OTf, Br, Cl
Y = OH, OR
R^1 = aromatic

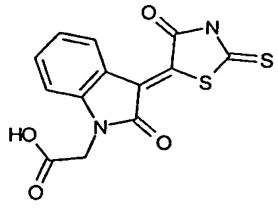
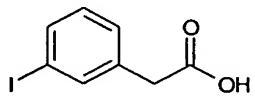
The above schemes could also apply where the building block attached to the DNA moiety is the second building block, and the subsequent moiety added is the third building block, or to link the initial building block to the linking moiety.

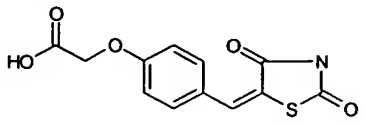
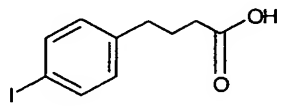
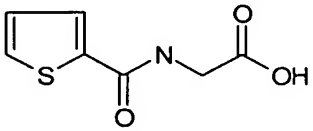
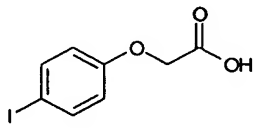
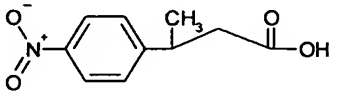
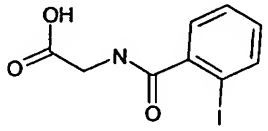
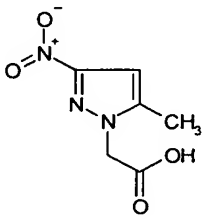
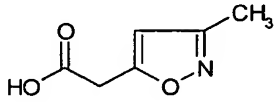
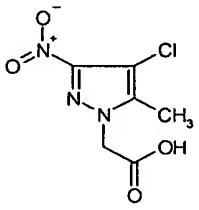
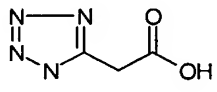
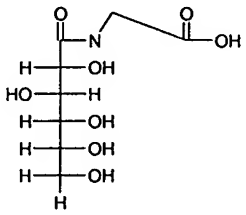
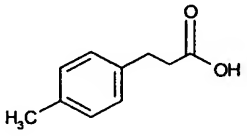
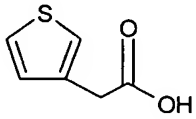
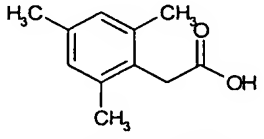
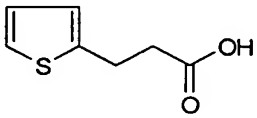
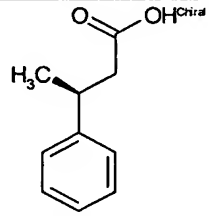
In one embodiment of the invention, the chemical moiety is formed from an initial and final building block, where the initial building block is an amino acid, and the final building block is a carboxylic acid. In particular, the collection of 20 initial building blocks is set out in Table 1:

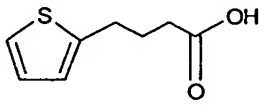
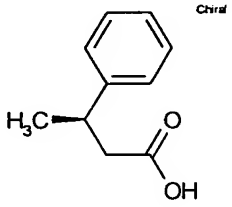
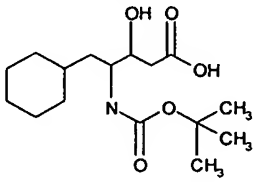
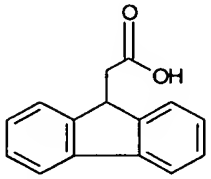
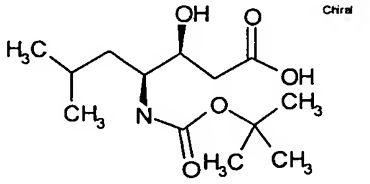
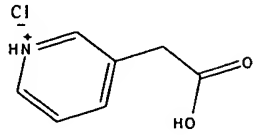
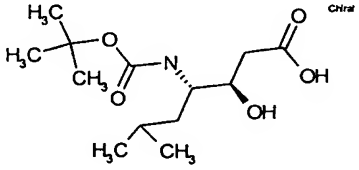
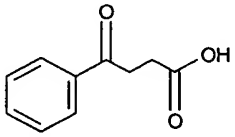
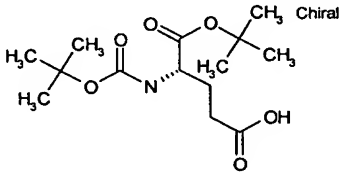
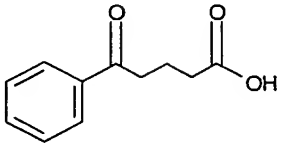
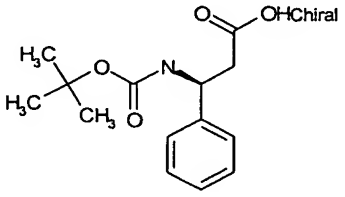
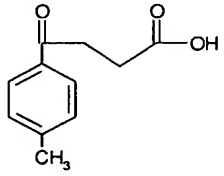
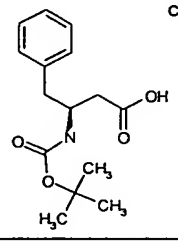
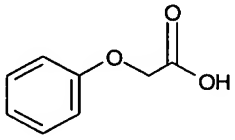
AA	Structure	AA	Structure
1		11	
2		12	
3		13	
4		14	
5		15	

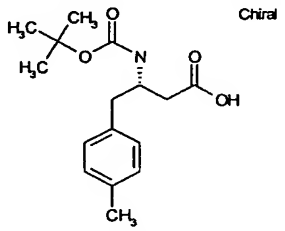
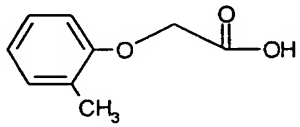
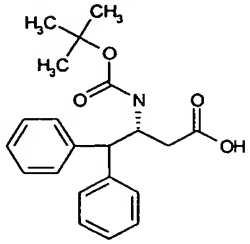
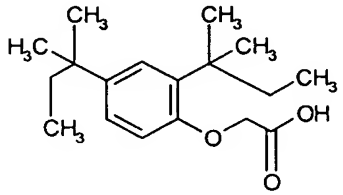
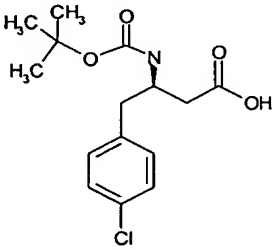
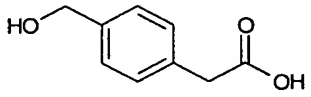
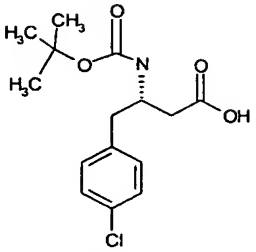
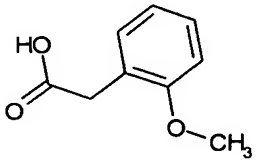
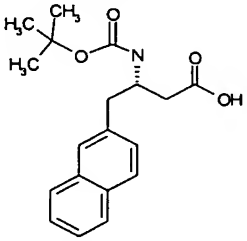
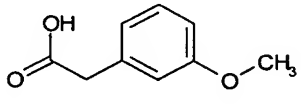
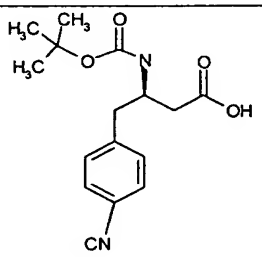
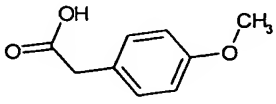
6		16	
7		17	
8		18	
9		19	
10		20	

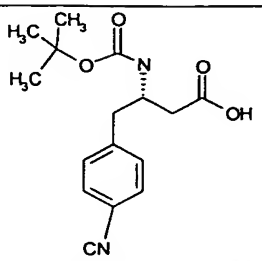
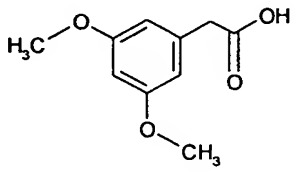
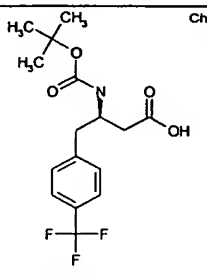
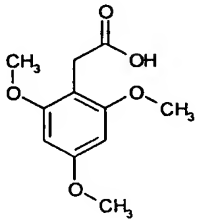
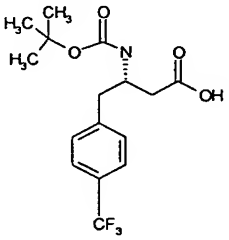
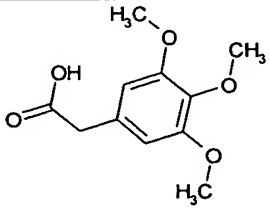
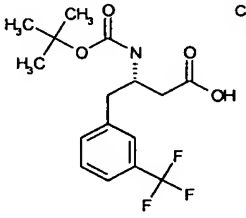
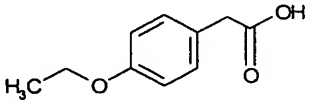
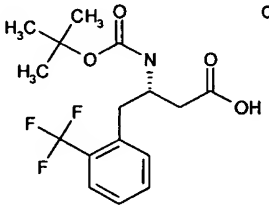
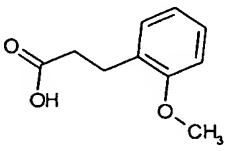
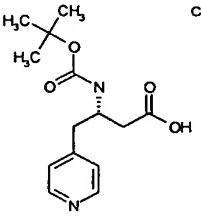
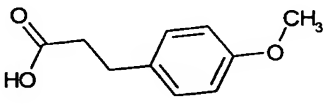
and the collection of 200 final building blocks is set out in Table 2:

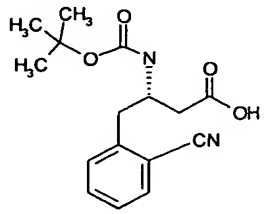
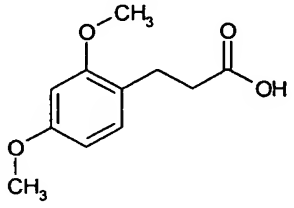
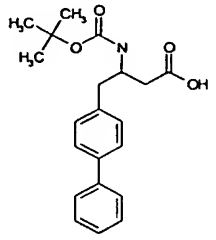
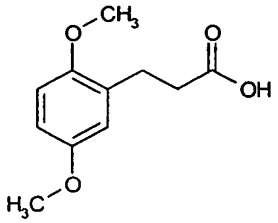
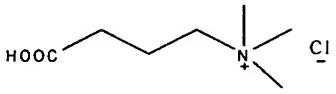
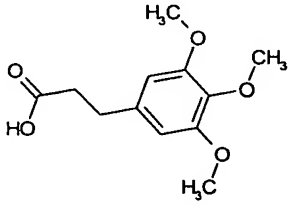
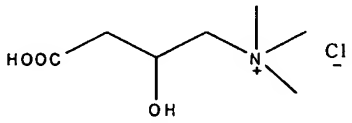
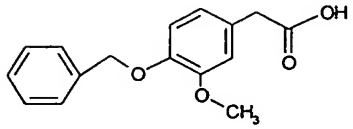
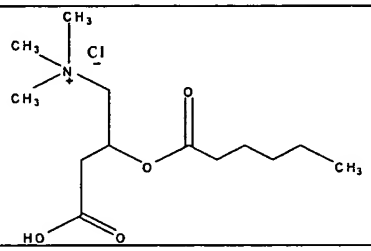
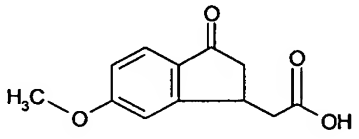
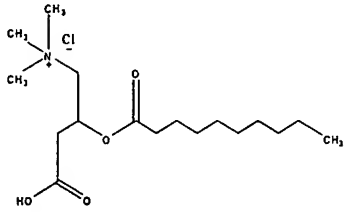
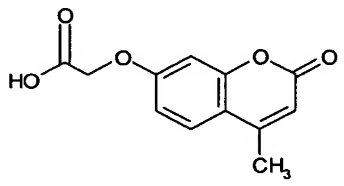
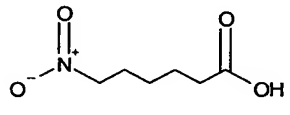
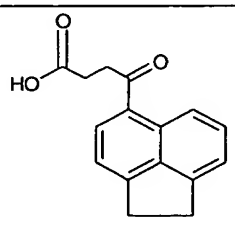
CA	Structure	CA	Structure
1		101	

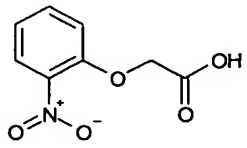
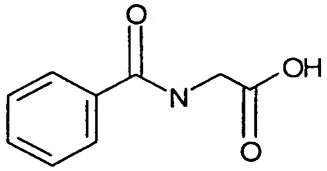
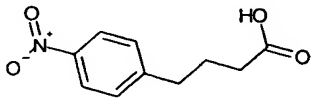
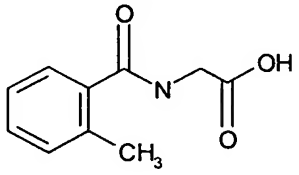
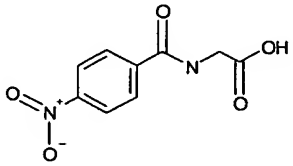
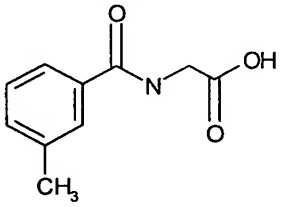
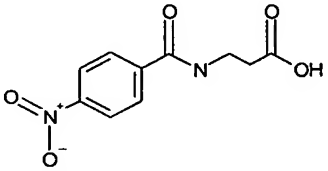
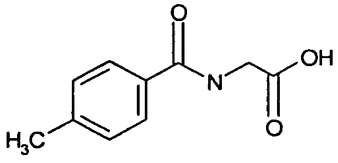
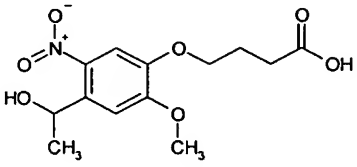
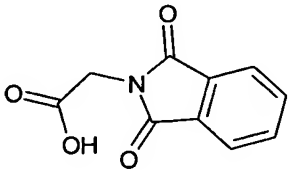
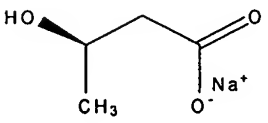
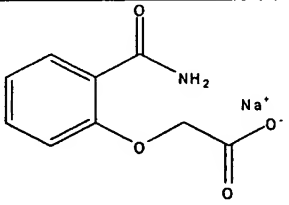
2		102	
3		103	
4		104	
5		105	
6		106	
7		107	
8		108	
9		109	

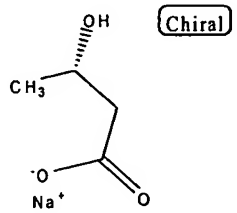
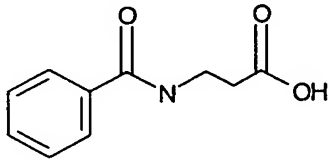
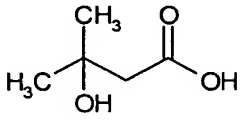
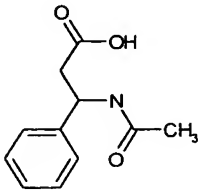
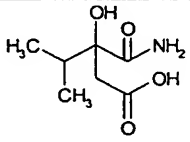
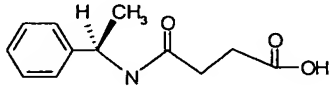
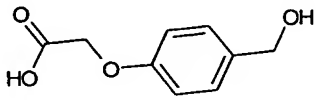
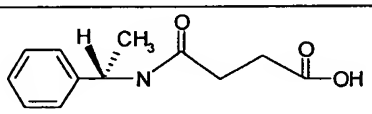
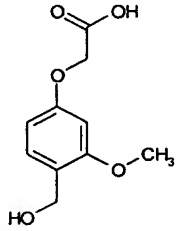
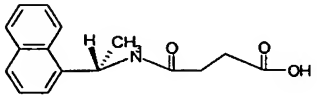
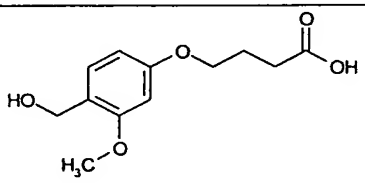
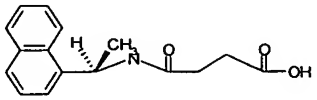
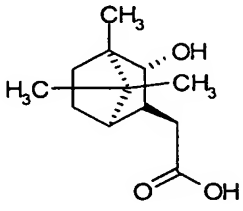
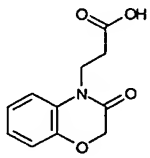
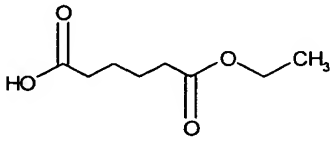
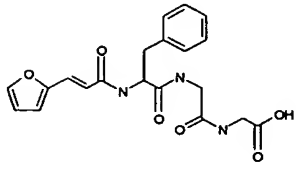
10		110	
11		111	
12		112	
13		113	
14		114	
15		115	
16		116	

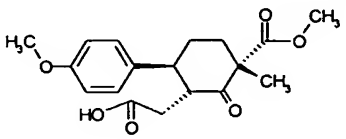
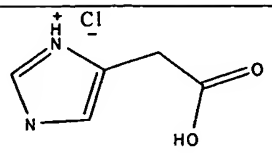
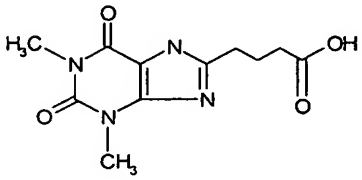
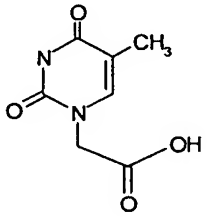
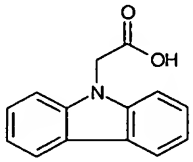
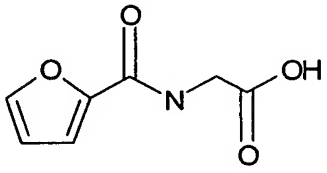
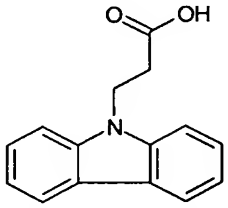
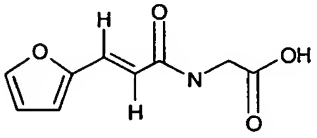
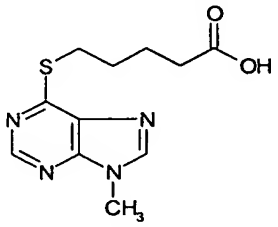
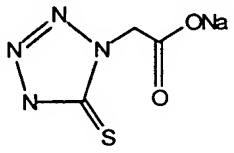
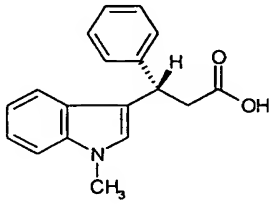
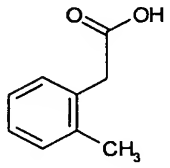
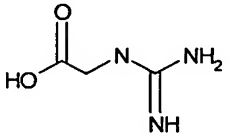
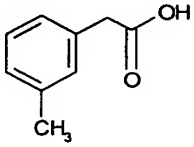
17		117	
18		118	
19		119	
20		120	
21		121	
22		122	

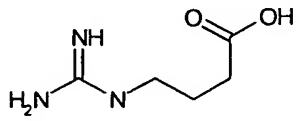
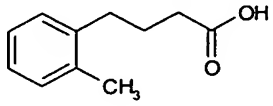
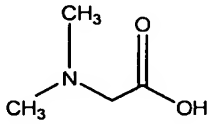
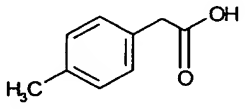
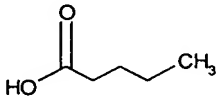
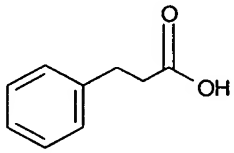
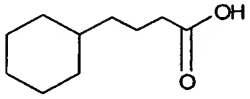
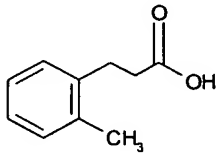
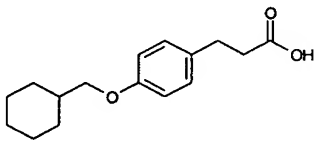
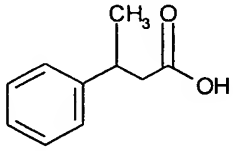
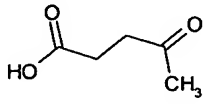
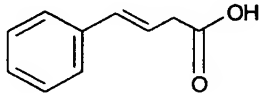
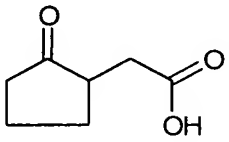
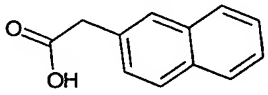
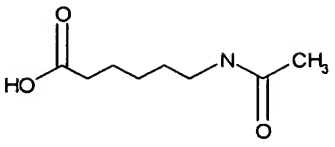
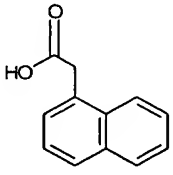
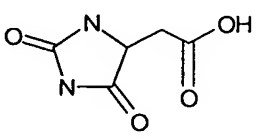
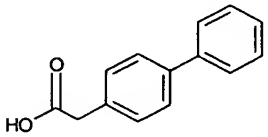
23		123	
24	Chiral 	124	
25		125	
26	Chiral 	126	
27	Chiral 	127	
28	Chiral 	128	

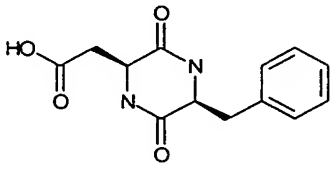
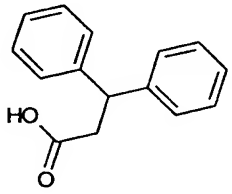
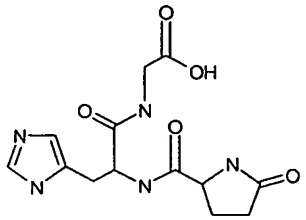
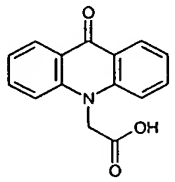
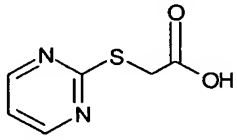
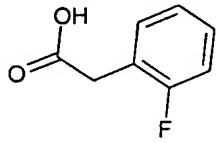
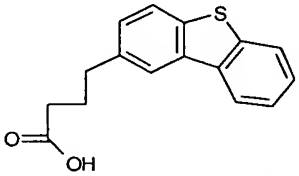
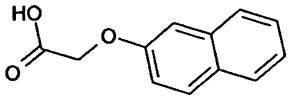
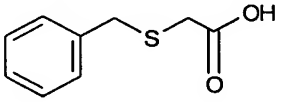
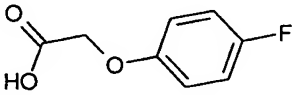
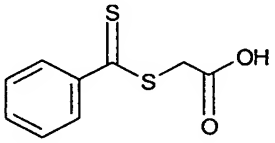
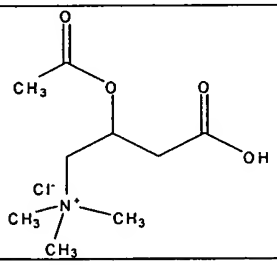
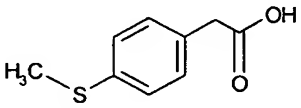
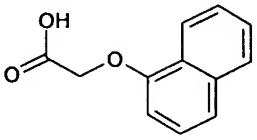
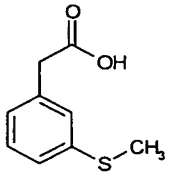
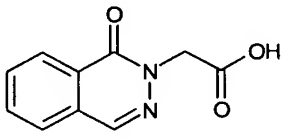
29		129	
30		130	
31		131	
32		132	
33		133	
34		134	
35		135	

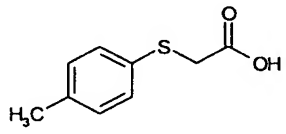
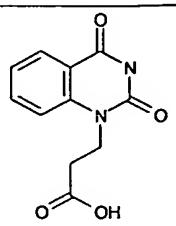
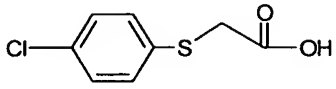
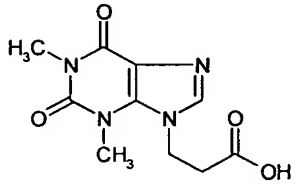
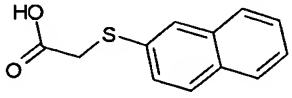
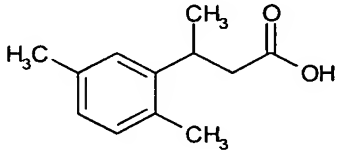
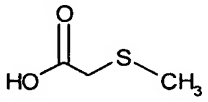
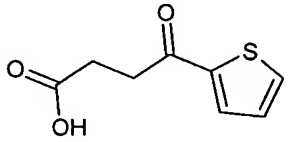
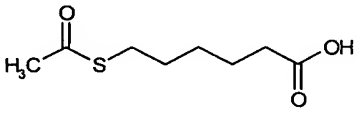
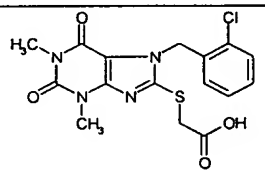
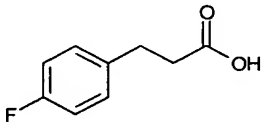
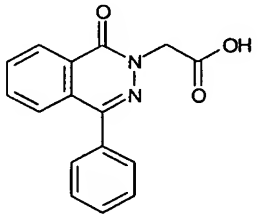
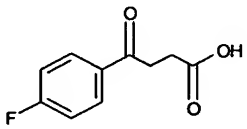
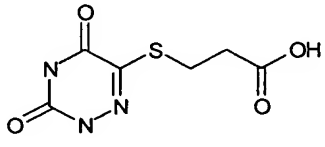
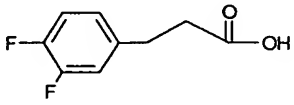
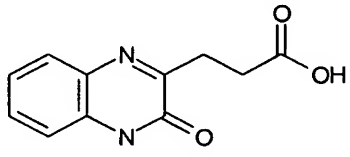
36		136	
37		137	
38		138	
39		139	
40		140	
41		141	

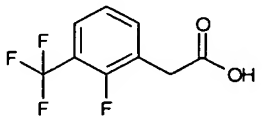
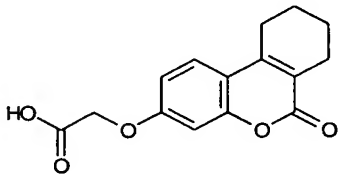
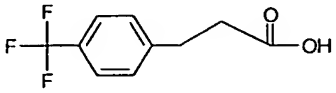
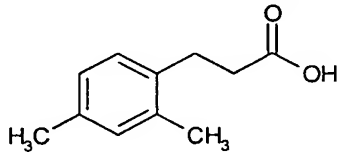
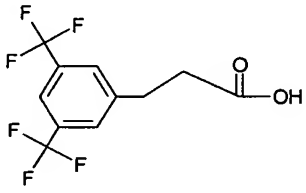
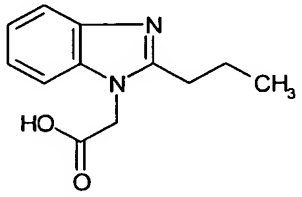
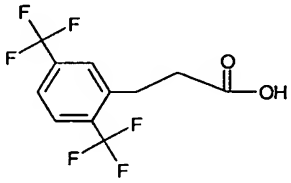
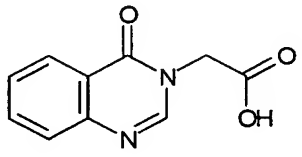
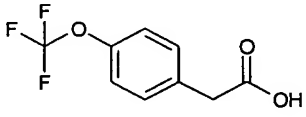
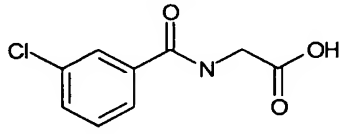
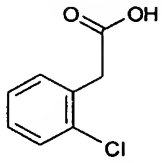
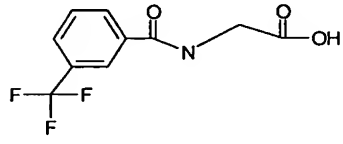
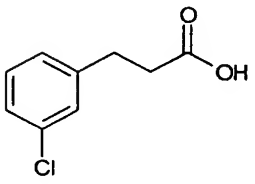
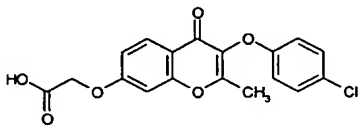
42		142	
43		143	
44		144	
45		145	
46		146	
47		147	
48		148	
49		149	

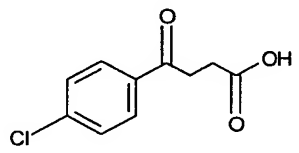
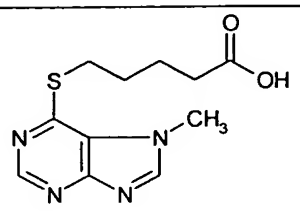
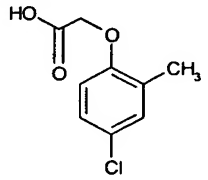
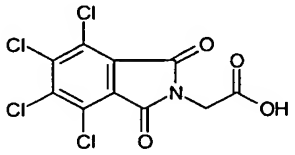
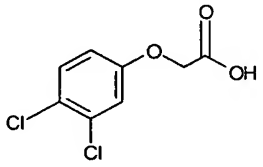
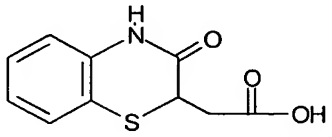
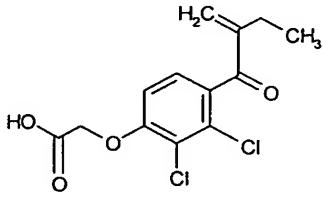
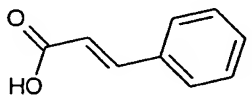
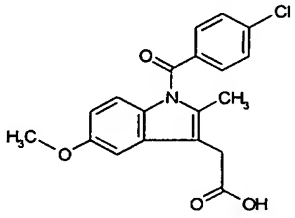
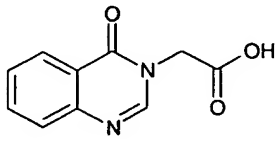
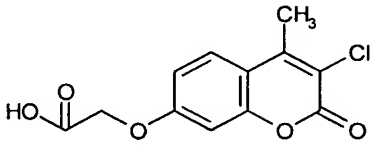
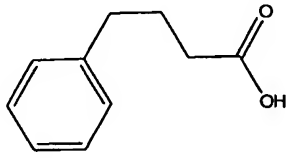
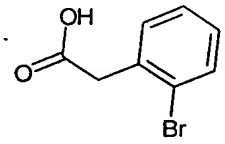
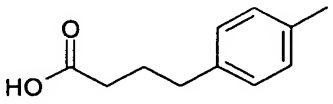
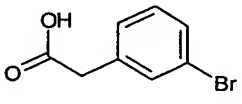
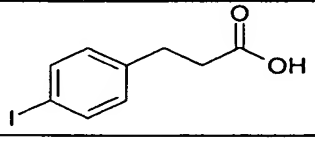
50		150	
51		151	
52		152	
53		153	
54		154	
55		155	
56		156	

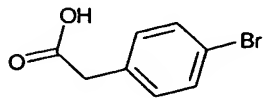
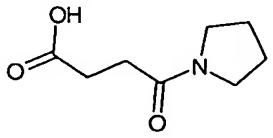
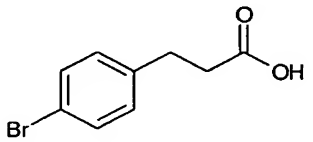
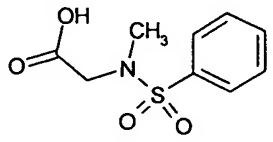
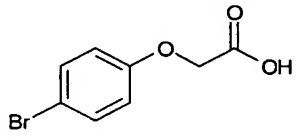
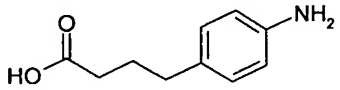
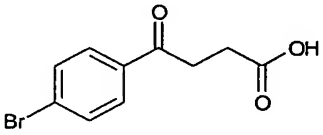
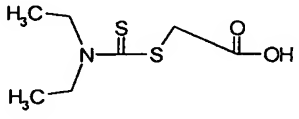
57		157	
58	 HCl	158	
59		159	
60		160	
61		161	
62		162	
63		163	
64		164	
65		165	

66		166	
67		167	
68		168	
69		169	
70		170	
71		171	
72		172	
73		173	

74		174	
75		175	
76		176	
77		177	
78		178	
79		179	
80		180	
81		181	

82		182	
83		183	
84		184	
85		185	
86		186	
87		187	
88		188	

89		189	
90		190	
91		191	
92		192	
93		193	
94		194	
95		195	
96		196	

97		197	
98		198	
99		199	
100		200	

Accordingly the present invention provides a DNA-encoded chemical library comprising members which comprise a chemical moiety, a linking moiety and a DNA moiety, wherein the chemical moiety is the reaction product of an initial building block and a final building block, the initial building blocks being selected from table 1 and the final building blocks being selected from table 2 where the library comprises members representing the reaction product of at least three initial building blocks and at least 5 final building blocks.

The library may comprise members representing the reaction product of at least five, ten fifteen or twenty initial building blocks and at least 10, 50, 100 or 200 final building blocks.

Although it is preferred that the library comprises members representing every possible reaction product of the initial and final building blocks, in some embodiments, the library comprises members representing at least 75, 90 or 95% of the possible reaction products.

Linker Moiety

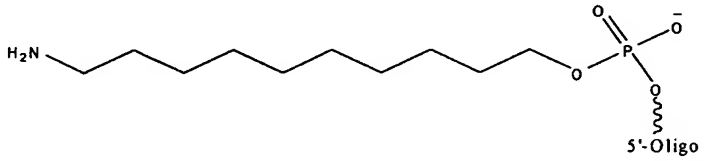
The linker moiety can be any moiety that performs the function of operatively linking the chemical moiety to the DNA moiety.

The linker moiety can vary in structure and length, and provide at least two features: (1) operative linkage to the chemical moiety and (2) operative linkage to the DNA moiety. As the nature of chemical linkages is diverse, any of a variety of chemistries may be utilized to effect the indicated operative linkages to both the chemical and DNA moieties, the nature of the

linkage is not considered an essential feature of this invention. The size of the linker moiety in terms of the length between the chemical and DNA moieties can vary widely, but for the purposes of the invention, need not exceed a length sufficient to provide the linkage functions indicated. Thus, a chain length of from at least one to about 20 atoms is preferred.

Typical linkers may be amino modifiers (such as 3'-Amino-Modifier C3, 3'-Amino-Modifier C6 dC, 3'-Amino-Modifier C6 dT, 3'-Amino-Modifier C7, 3'-PT-Amino-Modifier C3, 3'-PT-Amino-Modifier C6, 5'-Amino-dT-CE, 5'-Amino-Modifier 5, 5'-Amino-Modifier C12, 5'-Amino-Modifier C3, 5'-Amino-Modifier C6, Amino-Modifier C2 dT, Amino-Modifier C6 dA, Amino-Modifier C6 dC, Amino-Modifier C6 dG, Amino-Modifier C6 dT, Amino-Modifier C6-U, 5'-Amino Modifier C12), thiol modifiers (e.g. 3'-Thiol-Modifier C3 S-S, 5'-Thiol-Modifier C6, 3'-C6-Thiuol-Modifier S-S, 3'-C6-Thiol-Modifier, 5'-C6-Thiol-Modifier S-S, 5'-C6-Thiol-Modifier), carboxy modifiers (e.g. 3'-Carboxylate Photolabile C6, 5'-Carboxy-Modifier C10, Carboxy-dT), or aldehyde modifiers (e.g. 5'-Aldehyde-Modifier C2). These are readily commercially available

In the examples below, a 5'-Amino Modifier C12 was used as the linker molecule. This linker has the structure:



Thus in some embodiments of the present invention, the linker moiety is a 5'-amino modifier C12.

DNA moiety

The DNA moeity is double-stranded and comprises in the first aspect of the invention a first primer region, an initial coding region, a first annealing region, a final coding region and a second primer region, as well as optionally a middle coding region and second annealing region between the first annealing region and the final coding region. In the second aspect of the invention, the DNA moiety comprises a first primer region, an initial coding region, an annealing region, a middle coding region, a restriction/ligation region, a final coding region and a second primer region.

The first and second primer regions provide a means to produce a PCR-amplified duplex DNA fragment derived from the library member using PCR. The typical length of primer regions is between 16 and 28 nucleotides (in single stranded format), or base pairs (in double stranded

format). In double stranded format, one of the two DNA strands of the primer region can form a sequence specific dimer with a PCR primer at an appropriate hybridization temperature. Typical hibridization temperatures for the sequence specific hybridization of PCR primers to PCR primer regions is between 40 and 70°C. PCR primers can be longer than the hybridization region complementary to the primer region (at their respective 5' ends), e.g. for the introduction of additional sequences at the extremities of the PCR product that can be useful for later steps of the decoding process. In the examples below, PCR primers DEL_P1_A (5'-GCC TCC CTC GCG CCA TCA GGG AGC TTG TGA ATT CTG G-3') and DEL_P2_B (5'-GCC TTG CCA GCC CGC TCA GGT AGT CGG ATC CGA CCA C-3') were used. The sequences that are not underlined, are complementary to one of the 2 strands of the PCR primer regions on the double stranded library members.

Each coding region needs to contain sufficient nucleotides to uniquely identify the building block for which it is coding. For example, if the building block has 20 variants, the coding region needs to contain at least 3 nucleotides ($4^2 = 16$, $4^3 = 64$). The coding region may be longer than necessary. The benfit of employing coding regions that are longer than necessary is that they provide the opportunity to differentiate codes by more than just a single nucleotide difference, which gives more confidence in the decoding process. In the examples below, the initial building block (20 compounds) was encoded by 6 nucleotides, and the second building block (200 compounds) was encoded by 8 nucleotides.

The annealing regions need to be of a sufficient length to allow for good recognition and binding between the oligonulceotides. Typically, an annealing region will contain at least 10 nucleotides, and more preferably at least 18 nucleotides. Normally, the annealing region will contain no more than 28 nucleotides. If more than one annealing region is present, they should have different sequences. In the examples below, the annealing region contains 18 nucleotides.

As with hybridization with PCR primers, typical hybridization temperatures for the sequence specific annealing of two annealing regions are between 40°C and 70°C.

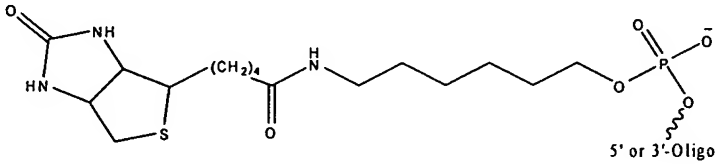
The restriction region used in the second aspect of the invention provides a site for restriction with a non-palindromic restriction enzyme that leaves a non-blunt (cohesive) end. This non-blunt end is matched by the ligation region used to couple the final coding region and second primer. The restriction region will contain preferably 6 nucleotides and normally no more then 8 nucleotides. The non-palindromic restriction site is required to prevent any non-specific ligation

on the final coding step. The non-palindromic restriction enzyme, may be, for example, BssSI (5'-CAC GAG-3'), BmgBI (5'-CAC GTC-3'), BbvCI (5'-CCT TCA GC-3'), BseYI (5'-CCC AGC-3') or BsrBI (5'-CCG CTC-3'). The enzyme restriction sequences are underlined.

In the examples below the restriction region contains 6 nucleotides and is specific for BssSI enzyme (5'-CAC GAG-3'). Thus, in embodiments of the invention the restriction region contains 6 nucleotides and is specific for BssSI enzyme (5'-CAC GAG-3').

The chemical modifier used in the first and second aspects of the invention may be a biotin moiety, or an imminobiotin moiety or any suitable chemical moiety that allows a selective capture by a macromolecular entity immobilized on a solid support, preferably high density coated streptavidin sepharose resin.

Thus, in embodiments of the invention the chemical modifier is either a 3'-Biotin-C6-modification or a 5'- Biotin-C6-modification. The modification has the structure:



The restriction region used in the third aspect of the invention provides a site for restriction with a restriction enzyme that leaves a non-blunt (cohesive) end. This non-blunt end is matched by the ligation region used to couple the final coding region and second primer. The restriction region will contain preferably 6 nucleotides and normally no more then 8 nucleotides. The restriction site is not required to be non-palindromic, as in the third aspect of the invention an excess of the final coding partially double stranded oligomers are used in the ligation. The restriction enzyme, may be, for example, BamHI (5'-GGA TCC-3'), or EcoRI (5'-GAA TTC-3'). The enzyme restriction sequences are underlined.

In example 8 below the restriction region contains 6 nucleotides and is specific for BamHI enzyme (5'-GGA TCC-3'). Thus, in embodiments of the third aspect of the invention the restriction region contains 6 nucleotides and is specific for BamHI enzyme (5'-GGA TCC-3').

Method steps

Coupling initial building block to initial coding single strand oligomer

The initial coding strand oligomer may be derivatised by a linking moiety using the appropriate conditions, as are well known in the art. In many embodiments, the initial coding strand oligomer may be puchased with the linking moiety already attached. The initial building block is reacted with the reactive terminus of the linking moiety under conditions which do not affect the conjugated oligomer. Different initial building blocks are conjugated to different oligonucleotides that bear coding regions specific for the initial building block they are coupled to.

Coupling building blocks

The building blocks may be coupled to each other using, for example, the chemistries set out above. As discussed, the reaction conditions used must be suitable to be used in the presence of single or double stranded DNA.

Annealing DNA

The annealing of the various oligonucleotides described above occurs by virtue of hybridization mediated by the complementary sequences of the annealing and complementary annealing regions. Incubating the mixture at an appropriate hybridization temperature (typically between 40 and 70°C) facilitates sequence specific annealing.

Use of DNA polymerase

By addition of desoxynucleotides (dNTPs) and a polymerase (e.g. Klenow polymerase) in a suitable buffer at the appropriate point in the method, and incubation at a suitable temperature (e.g. 37°C for Kenow polymerase), the DNA strands are elongated, forming a double stranded DNA segment.

Denaturing DNA and capture via chemical modifier

The double stranded DNA with suitable chemical modification is incubated with denaturing buffer (e.g. urea 4M aqueous) and eventually heated at 94°C. Following addition of the suitable macromolecular capturing agent immobilized on a solid support (e.g. high density coated streptavidin sepharose resin), the sample is incubated at an appropriate temperature, the supernatant separated by filtration and the desired single strand DNA isolated in the suitable buffer.

Treatment with restriction enzyme and capture via chemical modifier

Conjugates that are part of the second aspect of the invention containing a restriction site are treated with a restriction enzyme (e.g. BssSI) in a suitable buffer, and then incubated at the appropriate temperature for the enzyme (e.g. 37°C for BssSI). Two restriction fragments are

formed. On the one hand, a DNA fragment with the putative binding moiety conjugated to a DNA restriction fragments containing the coding tags, and on the other hand, a DNA restriction fragment with a chemical modifier, suitable for affinity capture. Subsequent addition of a suitable macromolecular ligand immobilized on a solid support (e.g. high density coated streptavidin sepharose resin), allows the separation by filtration of both restriction fragments.

Treatment with restriction enzyme

Conjugates that are part of the third aspect of the invention containing a restriction site are treated with a restriction enzyme (e.g. BamHI) in a suitable buffer, and then incubated at the appropriate temperature for the enzyme (e.g. 37°C for BamHI). Two restriction fragments are formed. On the one hand, a DNA fragment with the putative binding moiety conjugated to a DNA restriction fragments containing the coding tags, and on the other hand, a DNA restriction fragment. This DNA restriction fragment can be removed by chromatographic means.

Ligation of DNA

Ligation may be carried out by mixing of two sets of pre-hybridized suitable pairing oligonucleotides with cohesive ends, and a ligase enzyme (e.g. T4 ligase) in a suitable buffer, and incubation at appropriate temperature (e.g. 25°C for T4 ligase).

In the third aspect of the invention, an excess of oligonucleotides is used in the ligation step.

Uses of DNA-encoded libraries

The libraries of this invention provide a repertoire of chemical diversity such that each chemical moiety is linked to a DNA moiety that facilitates identification of the chemical moiety.

By the present screening methods, it is possible to identify optimised chemical structures that participate in binding interactions with a biological macromolecule by drawing upon a repertoire of structures randomly formed by the association of diverse building blocks without the necessity of either synthesising them one at a time or knowing their interactions in advance.

The invention therefore also contemplates a method for identifying a chemical moiety that participates in a preselected binding interaction between the chemical moiety and a biological macromolecule. The chemical moiety to be identified is represented by one of the members of a library of this invention, and the method comprises the following steps:

(a) a library according to the present invention is admixed with a preselected biological

macromolecule under binding conditions (i.e., a binding reaction admixture) for a time period sufficient for the biological macromolecule to interact with at least one member of the library and form a binding reaction complex;
(b) the binding reaction complex is then isolated from the library admixture to form an isolated complex;
(3) the sequence of the DNA moiety present in the isolated binding reaction complex is determined, this identifying the chemical moeity that participated in the binding reaction.

A typical biological macromolecule exhibiting a preselected binding interaction can be any of a variety of molecules (e.g. proteins) that bind selectively to another molecule, including antibodies to antigens, lectins to oligosaccharides, receptors to ligands, enzymes to substrates and the like mediators of molecular interactions. Therefore, a preselected binding interaction is defined by the selection of the biological macromolecule with which a library member is to bind.

Binding Reaction Admixtures
The admixture of a library of the invention with a biological macromolecule can be in the form of a heterogeneous or homogeneous admixture. Thus, the members of the library can be in the solid phase with the biological macromolecule present in the liquid phase. Alternatively, the biologically active molecule can be in the solid phase with the members of the library present in the liquid phase. Still further, both the library members and the biological macromolecule can be in the liquid phase.

Binding conditions are those conditions compatible with the known natural binding function of the biological macromolecule. Those compatible conditions are buffer, pH and temperature conditions that maintain the biological activity of the biological macromolecule, thereby maintaining the ability of the molecule to participate in its preselected binding interaction. Typically, those conditions include an aqueous, physiologic solution of pH and ionic strength normally associated with the biological macromolecule of interest.

For example, where the binding interaction is to identify a member in the library able to bind an antibody molecule, the preferred binding conditions would be conditions suitable for the antibody to immunoreact with its immunogen, or a known immunoreacting antigen. For a receptor molecule, the binding conditions would be those compatible with measuring receptor-ligand interactions.

A time period sufficient for the admixture to form a binding reaction complex is typically that

length of time required for the biological macromolecule to interact with its normal binding partner under conditions compatible with interaction. Although the time periods can vary depending on the molecule, admixing times are typically for at least a few minutes, and usually not longer than several hours, although nothing is to preclude using longer admixing times for a binding reaction complex to form.

A binding reaction complex is a stable product of the interaction between a biological macromolecule and a bifunctional molecule of this invention. The product is referred to as a stable product in that the interaction is maintained over sufficient time that the complex can be isolated from the rest of the members of the library without the complex becoming significantly disassociated.

Isolation of a Library Member from the Binding Reaction Admixture

A binding reaction complex is isolated from the binding reaction admixture by any separation means that is selective for the complex, thereby isolating that library member, or members, which hase/have bound to the biological macromolecule. There are a variety of separation means, depending on the status of the biological macromolecule.

For example, the biological macromolecule can be provided in admixture in the form of a solid phase reagent, i.e., affixed to a solid support, and thus can readily be separated from the liquid phase, thereby removing the majority of library members. Separation of the solid phase from the binding reaction admixture can optionally be accompanied by washes of the solid support to rinse library members having lower binding affinities off of the solid support.

Alternatively, for a homogeneous liquid binding reaction admixture, a secondary binding means specific for the biological macromolecule can be utilized to bind the molecule and provide for its separation from the binding reaction admixture.

For example, an immobilized antibody immunospecific for the biological macromolecule can be provided as a solid phase-affixed antibody to the binding reaction admixture after the binding reaction complex is formed. The immobilized antibody immunoreacts with the biological macromolecule present in the binding reaction admixture to form an antibody-biological macromolecule immunoreaction complex. Thereafter, by separation of the solid phase from the binding reaction admixture, the immunoreaction complex, and therefor any binding reaction complex, is separated from the admixture to form isolated library member.

Alternatively, a binding means can be operatively linked to the biological macromolecule to facilitate its retrieval from the binding reaction admixture. Exemplary binding means are one of the following high affinity pairs: biotin-avidin, protein A-Fc receptor, ferritin-magnetic beads, and the like. Thus, the biological macromolecule is operatively linked (conjugated) to biotin, protein A, ferritin and the like binding means, and the binding reaction complex is isolated by the use of the corresponding binding partner in the solid phase, e.g., solid-phase avidin, solid-phase Fc receptor, solid phase magnetic beads and the like.

The use of solid supports on which to operatively link proteinaceous molecules is generally well known in the art. Useful solid support matrices are well known in the art and include cross-linked dextran such as that available under the tradename SEPHADEX from Pharmacia Fine Chemicals (Piscataway, N.J.); agarose, borosilicate, polystyrene or latex beads about 1 micron to about 5 millimeters in diameter, polyvinyl chloride, polystyrene, cross-linked polyacrylamide, nitrocellulose or nylon-based webs such as sheets, strips, paddles, plates microtiter plate wells and the like insoluble matrices.

Determining the Identifier Sequence

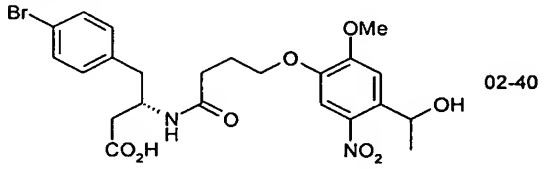
The DNA moieties of library members that bind to the target biological molecules are amplified by polymerase chain reaction (PCR), which allows very low amounts of template DNA to be detected. Subsequent decoding of the enriched DNA uses either DNA sequencing or hybridization to oligonucleotide microarrays, depending on the architecture of the library and its size.

A preferred method for decoding is the use of high throughput sequencing methods, such as the 454-Roche Genome Sequencer system. For sequencing with the 454-Roche Genome Sequencer system, PCR products have to contain suitable adaptor sequences at their extremities (calles adaptor sequence A and B), which can be either added after a PCR reaction by ligation, or they can be incorporated in the PCR reactions, if the PCR primers contain on their 5'-ends sequences corresponding to an adaptor region. For PCR amplification of the DNA moieties in the examples below, PCR primers DEL_P1_A (5'-GCC TCC CTC GCG CCA TCA GGG AGC TTG TGA ATT CTG G-3') and DEL_P2_B (5'-GCC TTG CCA GCC CGC TCA GGT AGT CGG ATC CGA CCA C-3') were used. The sequences that are not underlined, are complementary to one of the 2 strands of the PCR primer regions in the compounds. The underlined sequence of DEL_P1_A corresponds to the sequence of adaptor region A, and the underlined sequence of DEL-P2_B corresponds to adaptor sequence B.

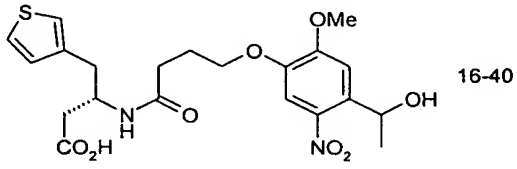
The next step of a particular sequencing process is the annealing of PCR amplicons on DNA Capture Beads, emulsification of beads and PCR reagents in water-in-oil microreactors, and clonal emPCR amplification inside these microreactors. After breaking of the emulsion, the Capture beads are mixed with Enzyme Beads, and loaded on a PicoTiterPlate. Pyrosequencing allows the recording of individual sequences for each DNA species displayed at Capture Beads, trapped in the wells of PicoTiterPlates. This allows the parallel sequencing of a vast amount (typically more than 100,000 per PicoTiterPlate) of individual DNA species at a time. With further improvement of the sequencing technology, it will be possible to sequence more than 1,000,000 individual DNA species at a time.

IgG binders

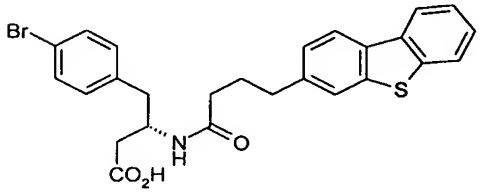
By means of the work carried out by the inventors, which is described in the examples below, certain chemical moieties have been identified which bind to IgG. These moieties are those derived from:



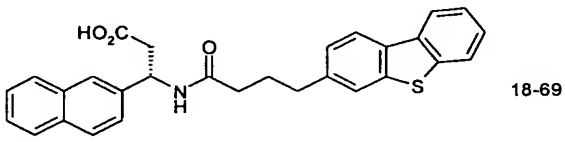
02-40



16-40



02-69

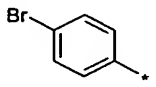


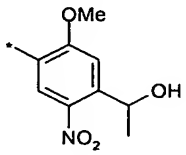
18-69

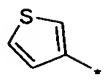
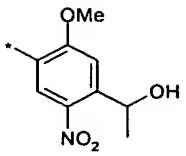
Compound 02-40 and 16-40 once bound to a solid support via the acid group have been shown in the examples below to act as affinity chromatography resins for IgG Cy5 labelled.

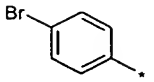
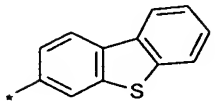
Accordingly a first further aspect of the present invention provides a compound selected from 02-40, 16-40 02-69 and 18-69. These compounds may used as affintity ligands in an affinity chromatography resin for IgG.

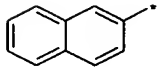
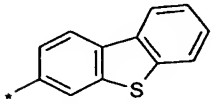
A second further aspect of the present invention provides an affinity chromatography resin for IgG comprising as the affinity ligand a moiety comprising a pair of constituent moieties being:

a) 

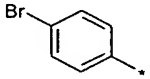
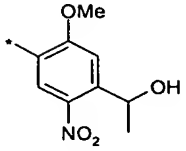


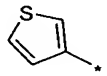
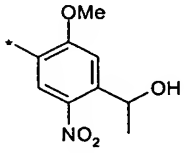
b)  

c)  

d)  

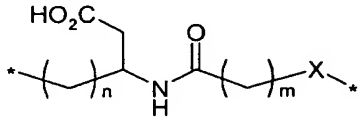
More preferably the pair of constituent moieties are:

a)  

b)  

The constituent moieties are linked together and to the sold support by a chemical linking group.

The chemical linking group may be of the formula:



wherein X is selected from O or CH_2;

n is 0, 1 or 2; and

m is 2, 3 or 4.

In preferred embodiments of this aspect the affinity ligand is selected from 02-40, 16-40, 02-69 and 18-69, and more preferably 02-40 and 16-40.

A third further aspect of the invention relates to the use of the affinity chromatography resin of the second further aspect for the purification of IgG.

The affinity chromatograpy resin used to immobilize the IgG binding moieties may comprise an

agarose carbohydrate polymer, for example, Sepharose Fast Flow. A highly cross-linked matrix makes the support more rigid which in turn improves the pressure/flow characteristic. In order to allow the biomolecules a rapid and unhindered access to the immobilized affinity ligand, the particle size needs to be no higher than 180 μm and preferebly spherical in the range 45-165 μm. The swelling factor is preferably higher then 3 drained medium/g. Furthermore the resin needs a wide range of pH and chemical stability to prevent any side reactivity during the washing and elution in various conditions.

In an embodiment of the invention, the resin support is Sepharose 4 Fast Flow resin (GE Healthcare) with a particle size 45-165 μm, swelling factor 4-5 drained medium/g and a pH stability range between 3-11.

Examples

General Experimental Methods

Reagents Unless otherwise denoted, chemical compounds and proteins were from Sigma-Aldrich-Fluka (Buchs, Switzerland), resin for solid phase synthesis from Novabiochem (Laufelfingen, Switzerland), enzymes from New England Biolabs (Germany) and HPLC grade lyophilized oligonucleotides were from IBA GmbH (Göttingen Germany). SpinX columns were purchased from Corning Costar Incorporated (USA, cat.no 8162) and ion-exchange cartridges for DNA purification from Qiagen, (PCR purification cat.no 28104, Nucleotides removal cat.no 28306) and used according to the protocol described by the provider.

Example 1: Library Synthesis

Summary

A DNA-encoded chemical library was constructed consisting of 20 x 200 modules, joined together by the formation of an amide bond. Initially, 20 Fmoc-protected aminoacids were chemically coupled to 20 individual amino-tagged oligonucleotides. Following deprotection and HPLC purification, the 20 resulting DNA-encoded primary amines were coupled to 200 carboxylic acids, in order to generate a library of 4000 members. In order to ensure that each library member contained a different DNA code, a split-and-pool strategy was chosen, which minimizes the number of oligonucleotides needed for library construction. The 20 Fmoc-protected aminoacids covalently linked to individual single-stranded oligonucleotides were mixed and aliquoted in 200 reaction vessels, prior to the coupling step with the 200 different carboxylic acids (one per well). The identity of the carboxylic acids used for the coupling reactions was encoded by performing an annealing step with individual oligonucleotides, partially complementary to the first oligo carrying the chemical modification. A successive

Klenow polymerization step yielded double stranded DNA fragments, each of which contained two identification codes (one corresponding to the initial 20 compounds and one corresponding to the 200 carboxylic acids). The 200 compound mixtures were then purified on an anion exchange cartridge and pooled. Model reactions performed prior to library construction had shown that the yields of the amide bond forming reaction ranged between 51% and 100%. The resulting DNA-encoded chemical library, containing 4'000 compounds, was dissolved in water, aliquoted at a total DNA concentration of 600 nM and stored at -20°C prior to further use.

Detailed Information

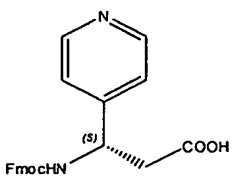
The individual organic compounds to be coupled to the 5' amino-modified 42-mer oligonucleotides were dissolved in DMSO stock solution (100mM), occasionally by further addition of water or diluted hydrochloric acid. All HPLC were performed on an XTerra Prep RP_{18} column (5µM, 10x150mm) using a linear gradient from 10% to 40% MeCN in 100mM TEAA, pH 7. All the LC-ESI-MS were performed on an XTerra RP_{18} column (5µM, 4.6x20mm) using a linear gradient from 0% to 50% MeOH over 1min in 400mM HFIP/5mM TEA. The mass spectra were measured from 900 to 2000 *m/z* by a Waters Quattro Micro instrument.

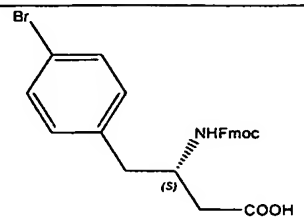
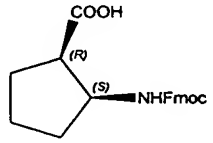
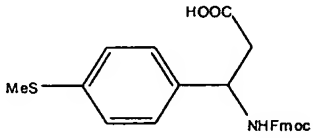
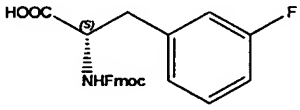
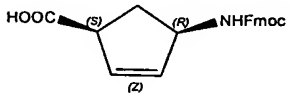
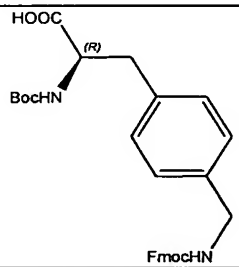
Coupling reactions of 20 fmoc-protected aminoacids

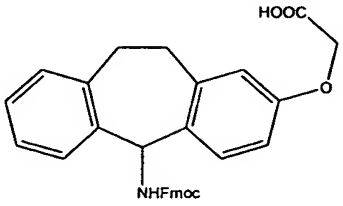
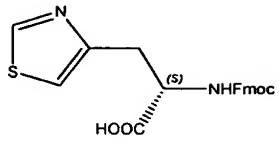
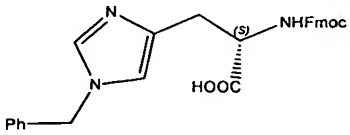
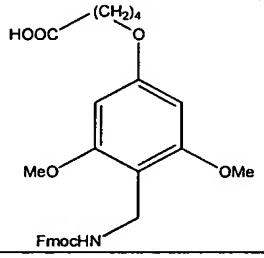
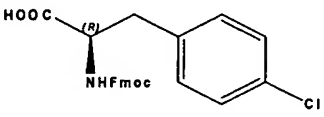
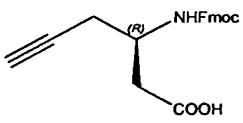
To a reaction volume of 310µL, containing 70%(v/v) DMSO/water, compounds were added to the respective final concentrations: fmoc-protected-aminoacids DMSO solution, 30mM; N-hydroxysulfosuccinimide (Fluka,) in DMSO, 10mM; N-ethyl-N'-(3-dimethylaminopropyl)-carbodiimide (Fluka) in DMSO, 4mM; triethylamine hydrochloride water solution, pH 9.0, 80mM; oligonucleotide (IBA) water solution, 50µM, (5'-GGAGCTTGTGAATTCTGGXXXXXXGGACGTGTGTGAATTGTC-3', XXXXXX unambiguously identifies the individual fmoc-protected aminoacid compounds).

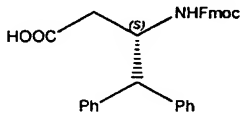
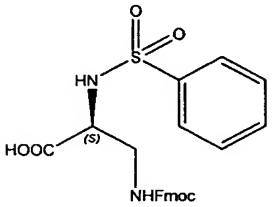
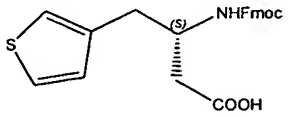
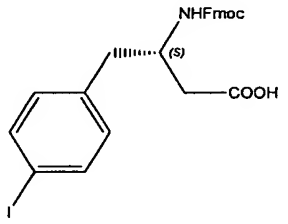
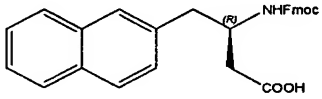
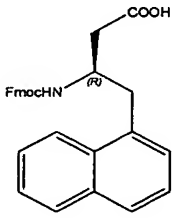
Table 1 lists the amino acids and the oligonucleotide code used to identify them:

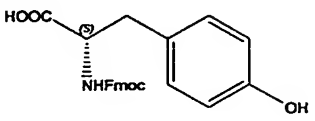
Table 1

AA	Structure	Name	Oligonucleotide Code					
1		(S)-3-(((9H-fluoren-9-yl)methoxy)carbonylamino)-3-(pyridin-4-yl)propanoic acid	A	T	C	T	T	A

2		3-(((9H-fluoren-9-yl)methoxy)carbonylamino)-4-(4-bromophenyl)butanoic acid	G	C	T	G	C	G
3		(1R,2S)-2-(((9H-fluoren-9-yl)methoxy)carbonylamino)cyclopentanecarboxylic acid	A	G	A	A	C	G
4		3-(((9H-fluoren-9-yl)methoxy)carbonylamino)-3-(pyridin-2-yl)propanoic acid	G	A	C	A	T	C
5		(S)-2-(((9H-fluoren-9-yl)methoxy)carbonylamino)-3-(3-fluorophenyl)propanoic acid	A	T	T	A	C	T
6		(1S,4R)-4-(((9H-fluoren-9-yl)methoxy)carbonylamino)cyclopent-2-enecarboxylic acid	A	C	G	G	C	A
7		(R)-3-(4-((((9H-fluoren-9-yl)methoxy)carbonylamino)methyl)phenyl)-2-(tert-butoxycarbonylamino)propanoic acid	A	G	A	G	A	A

8		Acetic acid, [[5-[[(9H-fluoren-9-ylmethoxy)carbonyl]amino]-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-2-yl]oxy]	T	C	C	A	A	A
9		(S)-2-(((9H-fluoren-9-yl)methoxy)carbonylamino)-3-(thiazol-4-yl)propanoic acid	T	C	G	A	T	C
10		(S)-2-(((9H-fluoren-9-yl)methoxy)carbonylamino)-3-(1-benzyl-1H-imidazol-4-yl)propanoic acid	T	C	C	G	G	C
11		5-(4-((((9H-fluoren-9-yl)methoxy)carbonylamino)methyl)-3,5-dimethoxyphenoxy)pentanoic acid	C	G	T	G	C	A
12		(R)-2-(((9H-fluoren-9-yl)methoxy)carbonylamino)-3-(4-chlorophenyl)propanoic acid	G	G	G	T	A	A
13		(R)-3-(((9H-fluoren-9-yl)methoxy)carbonylamino)hex-5-ynoic acid	C	C	C	T	C	C

14		(S)-3-(((9H-fluoren-9-yl)methoxy)carbonylamino)-4,4-diphenylbutanoic acid	T	C	T	C	C	A
15		(S)-3-(((9H-fluoren-9-yl)methoxy)carbonylamino)-2-(phenylsulfonamido)propanoic acid	C	A	A	G	C	T
16		(S)-3-(((9H-fluoren-9-yl)methoxy)carbonylamino)-4-(thiophen-3-yl)butanoic acid	G	C	A	C	T	G
17		(S)-3-(((9H-fluoren-9-yl)methoxy)carbonylamino)-4-(4-iodophenyl)butanoic acid	A	C	G	A	A	T
18		(R)-3-(((9H-fluoren-9-yl)methoxy)carbonylamino)-4-(naphthalen-2-yl)butanoic acid	T	A	T	C	A	G
19		(R)-3-(((9H-fluoren-9-yl)methoxy)carbonylamino)-4-(naphthalen-1-yl)butanoic acid	T	G	A	A	A	T

20		(S)-2-(((9H-fluoren-9-yl)methoxy)carbonylamino)-3-(4-hydroxyphenyl)propanoic acid	G	T	T	A	G	T

All coupling reactions were stirred overnight at room temperature; residual activated species were then quenched by addition of piperidine (Fluka) 500mM DMSO. Prior to HPLC purification 500µL of 100mM TEAA, pH 7.0, was added to the reaction mixture. The reactions were then purified by HPLC and the desired samples were redissolved in 100µL of water and an amount of 10µL was analyzed by LC-ESI-MS. The samples showed the expected desired fmoc-deprotected product. Typical coupling yields were >54% overall. 4.0 nmol of each DNA conjugated compound were pooled together to generate a 20 member DNA encoded sub-library.

Coupling reactions of 200 carboxylic acids

To a reaction volume of 310µL, containing 70% (v/v) DMSO/water, compounds were added to the respective final concentrations: DMSO-dissolved carboxylic acid, 30mM; N-hydroxysulfosuccinimide (Fluka) in DMSO, 10mM; N-ethyl-N'-(3-dimethylaminopropyl)-carbodiimide (Fluka) in DMSO, 4mM; triethylaminehydrochloride, pH9.0 water solution, 80mM; DNA-oligonucleotide sub-library pool, 1.5µM. All coupling reactions were stirred overnight at room temperature; residual activated species were then quenched by addition of 50µL Tris-Cl buffer, 500mM pH 9.0. The mixture was allowed to quantitatively precipitate by sequential addition of 25µL of 1M acetic acid, 12.5µL of 3M sodium acetate buffer, pH 4.7 and 500µL ethanol followed by 2 hours incubation at -23°C. The mixture was centrifuged and the resulting oligonucleotide pellet was washed with ice-cold 90% (v/v) ethanol and then dissolved in 100µL millipore water. Test coupling reactions were also performed with the reaction conditions described above; using model 42mer 5'-fmoc-deprotected aminoacids-oligonucleotide conjugate and model carboxylic acids. The reactions were analysed by HPLC and the masses of the reacted oligonucleotides detected by LC-ESI-MS. Typical HPLC coupling yields on this step were >58% with purity >46%.

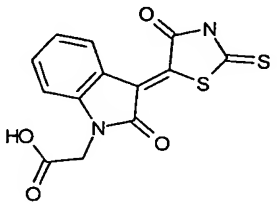
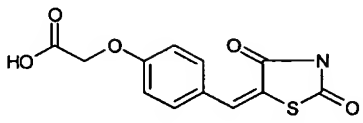
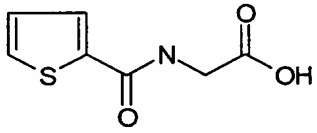
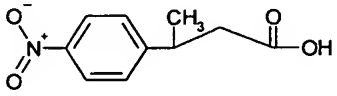
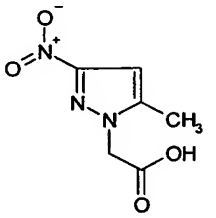
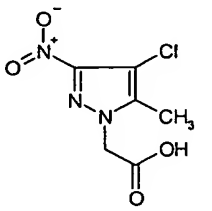
Polymerase Klenow encoding of 200 carboxylic acids reactions

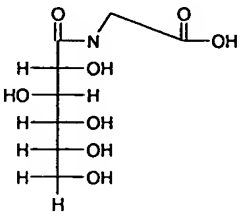
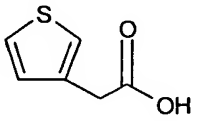
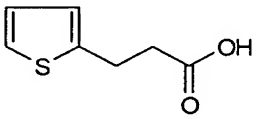
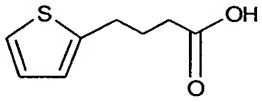
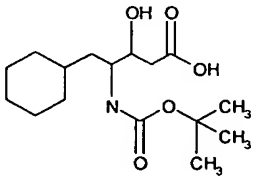
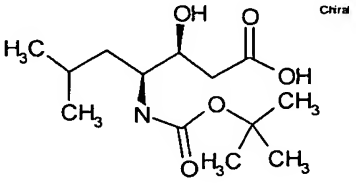
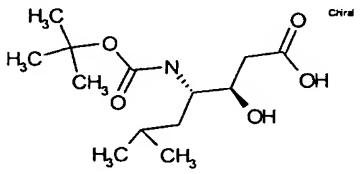
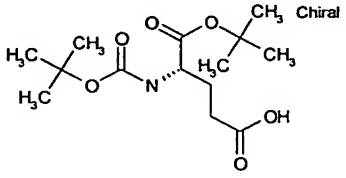
To a reaction volume of 50µL, reagents were added to the respective final concentrations: pool of compounds 320nM, 44mer oligonucleotide (5'-GTAGTCGGATCCGACCACXXXXXXXXGACAATTCACACACGTCC-3', XXXXXXXX

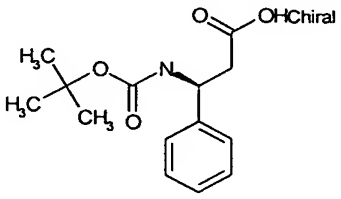
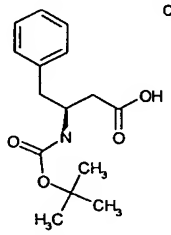
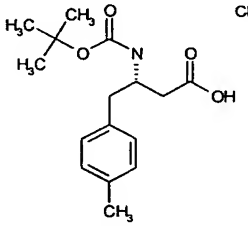
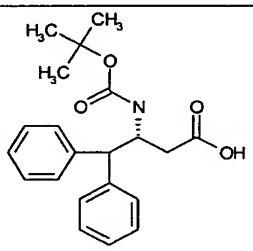
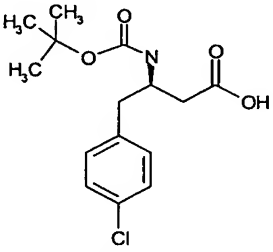
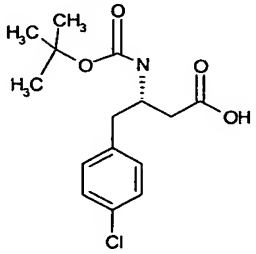
unambiguously identifies the individual carboxylic acid compound, IBA) 600nM, Klenow buffer (NEB, cat.no B7002S), dNTPs (Roche, cat.no 11969064001), 0.5mM, Klenow Polymerase enzyme (NEB, cat.no M0210L), 5 units. All the Klenow polymerization were incubated at 37°C for 1 hour and then purified on ion-exchange cartridge (Qiagen, cat.no 28306). All the 200 purified reactions were dissolved in 50µL of water and pooled to generate the 4000 member library (DEL4000) to a final oligonucleotide concentration of 300 nM.

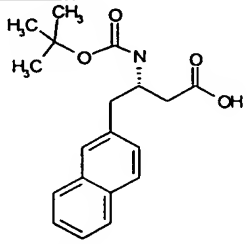
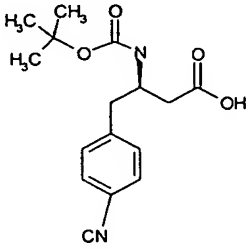
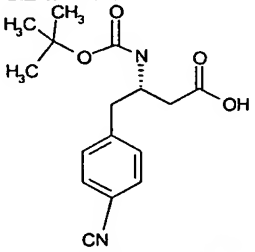
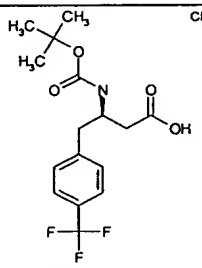
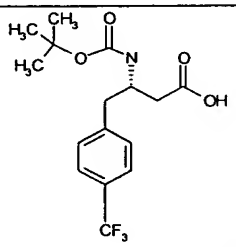
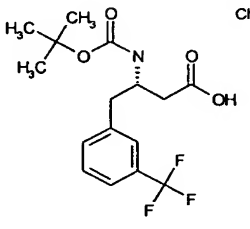
Table 2 lists the carboxylic acids and the oligonucleotide code used to identify them:

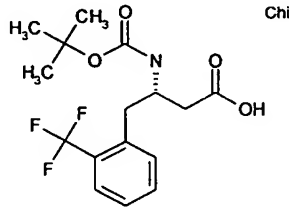
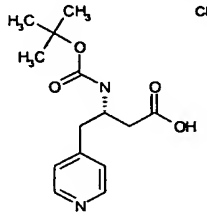
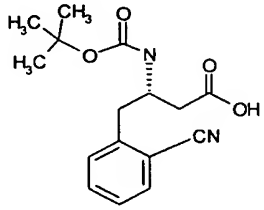
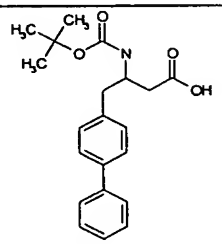
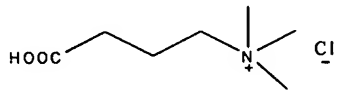
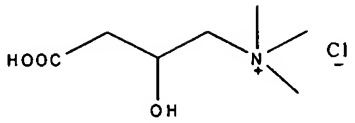
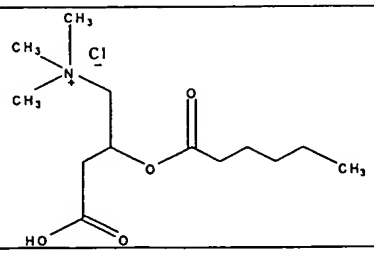
Table 2

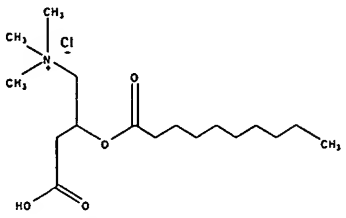
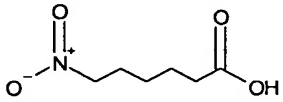
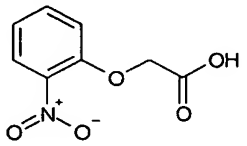
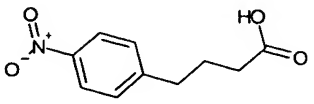
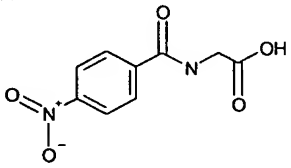
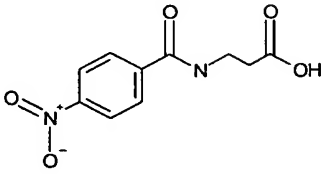
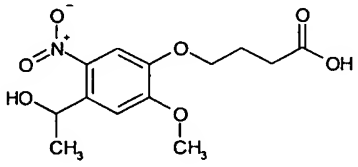
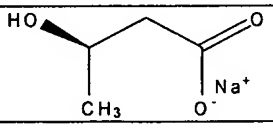
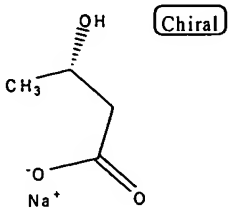
CA	Structure	Oligonucleotide Code							
1		T	T	T	T	T	T	T	T
2		G	G	G	G	T	T	T	T
3		C	C	C	C	T	T	T	T
4		A	A	A	A	T	T	T	T
5		A	C	G	T	G	T	T	T
6		C	A	T	G	G	T	T	T

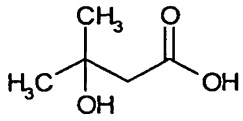
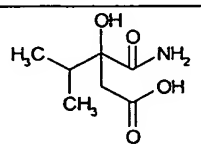
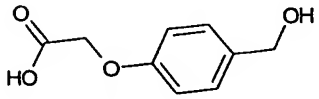
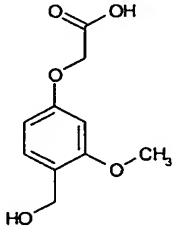
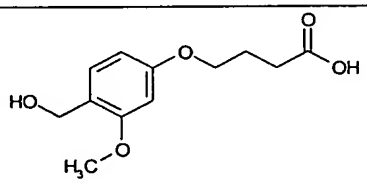
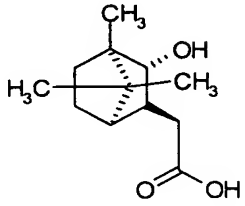
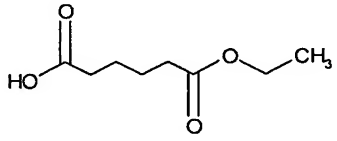
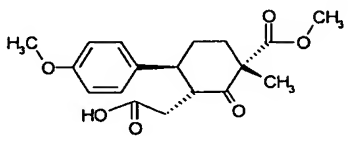
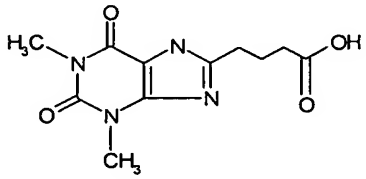
7		G	T	A	C	G	T	T	T
8		T	G	C	A	G	T	T	T
9		G	A	C	T	C	T	T	T
10		T	C	A	G	C	T	T	T
11		A	G	T	C	C	T	T	T
12		C	T	G	A	C	T	T	T
13		C	G	A	T	A	T	T	T
14		A	T	C	G	A	T	T	T

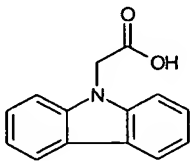
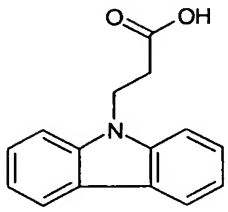
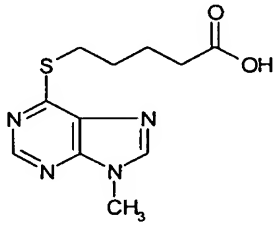
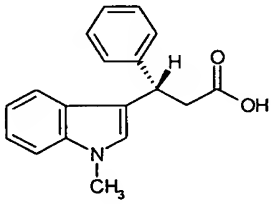
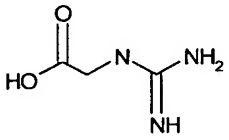
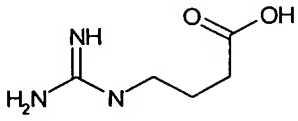
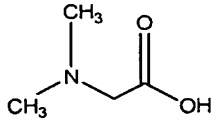
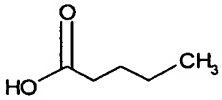
15		T	A	G	C	A	T	T	T
16		G	C	T	A	A	T	T	T
17		C	A	G	T	T	G	T	T
18		A	C	T	G	T	G	T	T
19		T	G	A	C	T	G	T	T
20		G	T	C	A	T	G	T	T

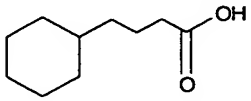
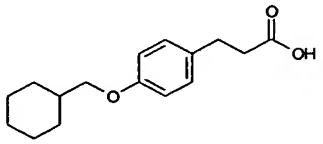
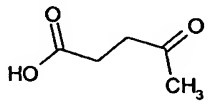
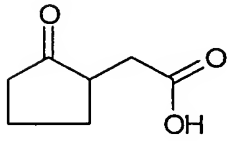
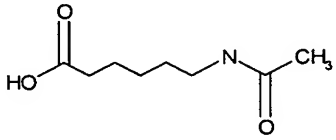
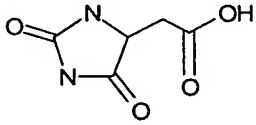
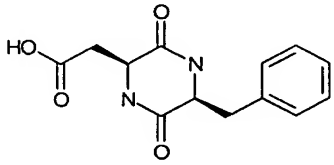
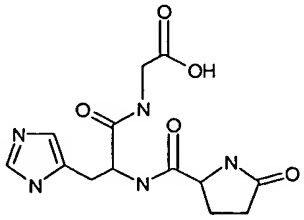
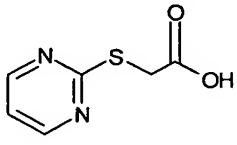
21		G	G	T	T	G	G	T	T
22		T	T	G	G	G	G	T	T
23		A	A	C	C	G	G	T	T
24	Chiral 	C	C	A	A	G	G	T	T
25		A	T	A	T	C	G	T	T
26	Chiral 	C	G	C	G	C	G	T	T

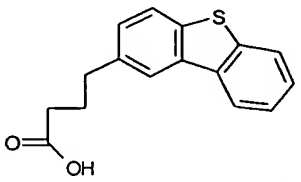
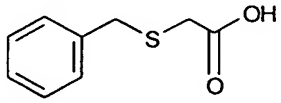
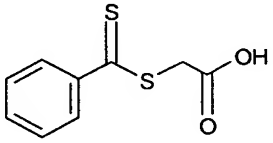
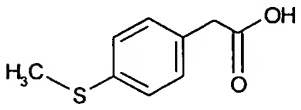
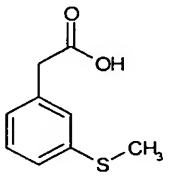
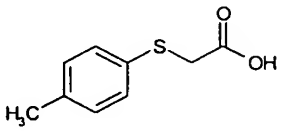
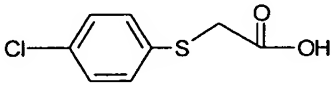
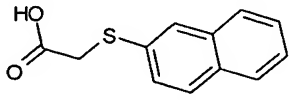
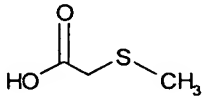
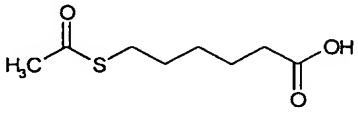
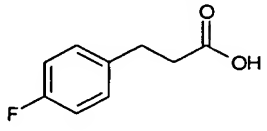
27	 Chiral	G	C	G	C	C	G	T	T
28	 Chiral	T	A	T	A	C	G	T	T
29		T	C	C	T	A	G	T	T
30		G	A	A	G	A	G	T	T
31		C	T	T	C	A	G	T	T
32		A	G	G	A	A	G	T	T
33		A	G	C	T	T	C	T	T

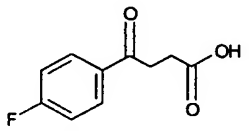
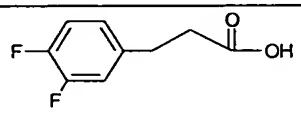
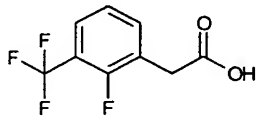
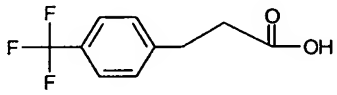
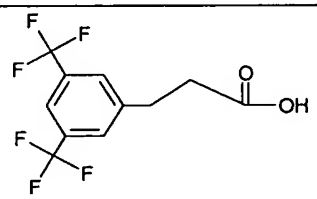
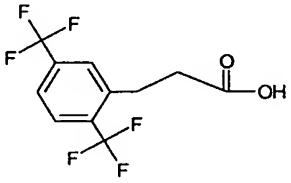
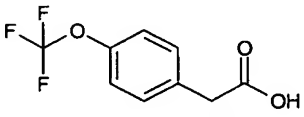
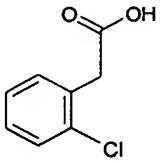
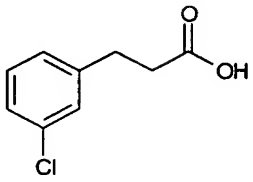
34		C	T	A	G	T	C	T	T
35		G	A	T	C	T	C	T	T
36		T	C	G	A	T	C	T	T
37		T	A	A	T	G	C	T	T
38		G	C	C	G	G	C	T	T
39		C	G	G	C	G	C	T	T
40		A	T	T	A	G	C	T	T
41		C	C	T	T	C	C	T	T
42		A	A	G	G	C	C	T	T

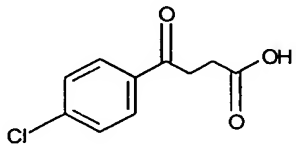
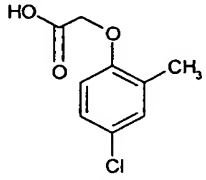
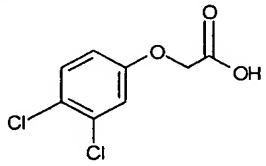
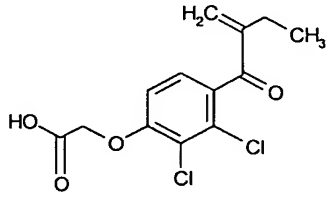
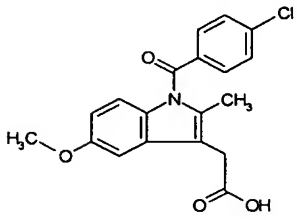
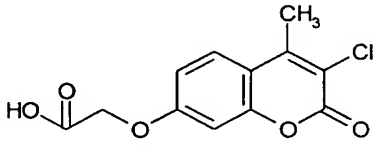
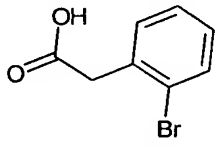
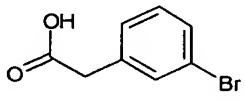
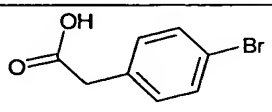
43		T	T	C	C	C	C	T	T
44		G	G	A	A	C	C	T	T
45		G	T	G	T	A	C	T	T
46		T	G	T	G	A	C	T	T
47		A	C	A	C	A	C	T	T
48		C	A	C	A	A	C	T	T
49		G	C	A	T	T	A	T	T
50		T	A	C	G	T	A	T	T
51		A	T	G	C	T	A	T	T

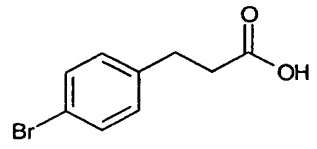
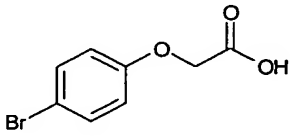
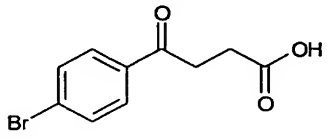
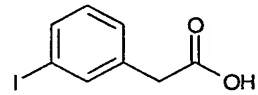
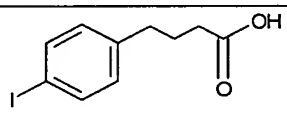
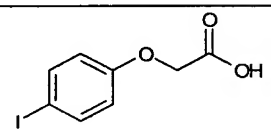
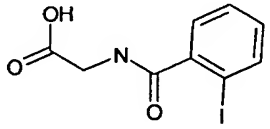
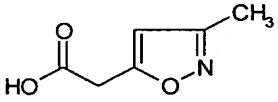
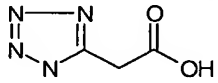
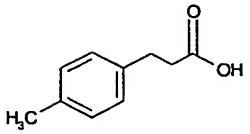
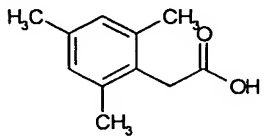
52		C	G	T	A	T	A	T	T
53		C	T	C	T	G	A	T	T
54		A	G	A	G	G	A	T	T
55		T	C	T	C	G	A	T	T
56		G	A	G	A	G	A	T	T
57		T	G	G	T	C	A	T	T
58	 HCl	G	T	T	G	C	A	T	T
59		C	A	A	C	C	A	T	T

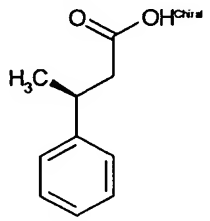
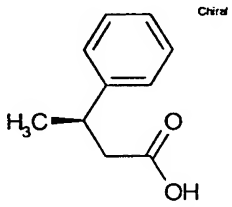
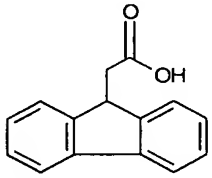
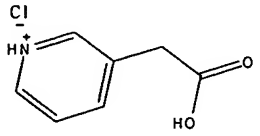
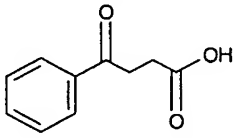
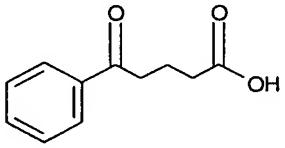
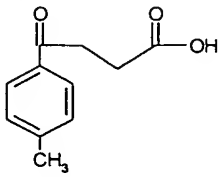
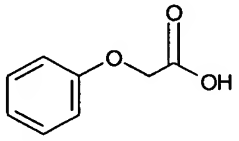
60		A	C	C	A	C	A	T	T
61		A	A	T	T	A	A	T	T
62		C	C	G	G	A	A	T	T
63		G	G	C	C	A	A	T	T
64		T	T	A	A	A	A	T	T
65		G	G	T	T	T	T	G	G
66		T	T	G	G	T	T	G	G
67		A	A	C	C	T	T	G	G
68		C	C	A	A	T	T	G	G

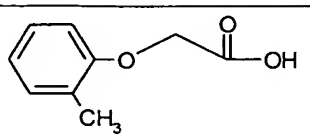
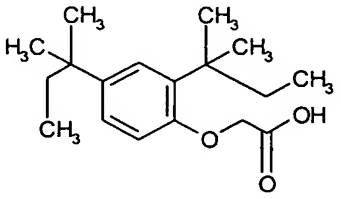
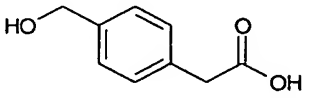
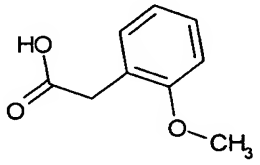
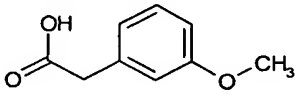
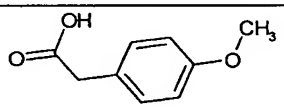
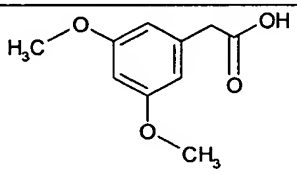
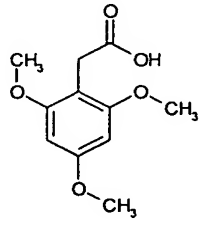
69		C	A	G	T	G	T	G	G
70		A	C	T	G	G	T	G	G
71		T	G	A	C	G	T	G	G
72		G	T	C	A	G	T	G	G
73		T	C	C	T	C	T	G	G
74		G	A	A	G	C	T	G	G
75		C	T	T	C	C	T	G	G
76		A	G	G	A	C	T	G	G
77		A	T	A	T	A	T	G	G
78		C	G	C	G	A	T	G	G
79		G	C	G	C	A	T	G	G

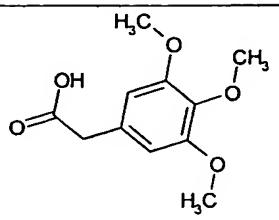
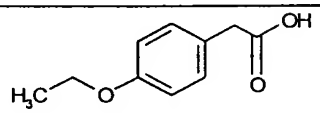
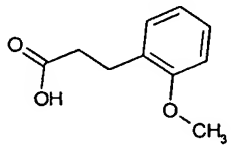
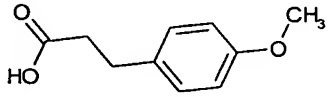
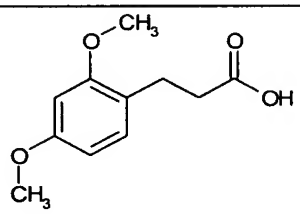
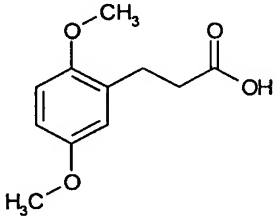
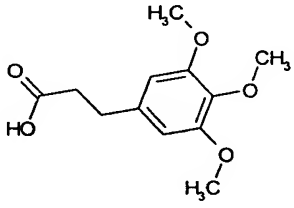
80		T	A	T	A	A	T	G	G
81		A	C	G	T	T	G	G	G
82		C	A	T	G	T	G	G	G
83		G	T	A	C	T	G	G	G
84		T	G	C	A	T	G	G	G
85		T	T	T	T	G	G	G	G
86		G	G	G	G	G	G	G	G
87		C	C	C	C	G	G	G	G
88		A	A	A	A	G	G	G	G

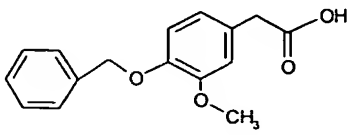
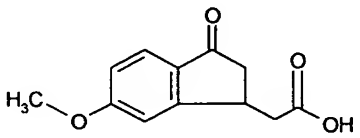
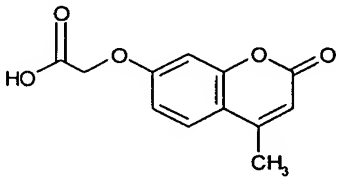
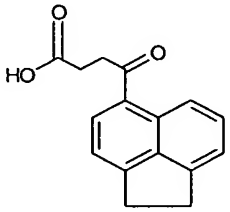
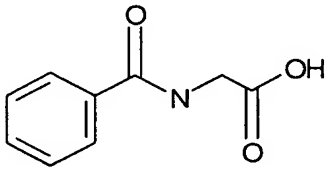
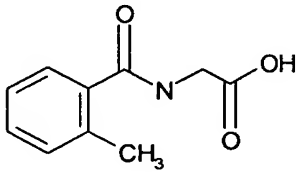
89		C	G	A	T	C	G	G	G
90		A	T	C	G	C	G	G	G
91		T	A	G	C	C	G	G	G
92		G	C	T	A	C	G	G	G
93		G	A	C	T	A	G	G	G
94		T	C	A	G	A	G	G	G
95		A	G	T	C	A	G	G	G
96		C	T	G	A	A	G	G	G
97		C	T	C	T	T	C	G	G

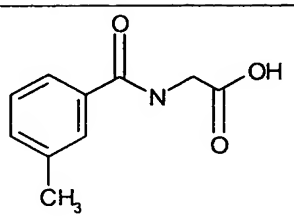
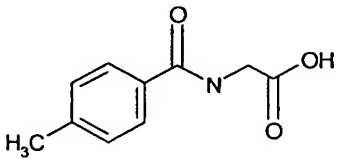
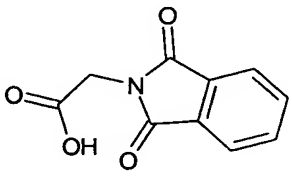
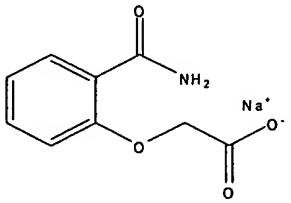
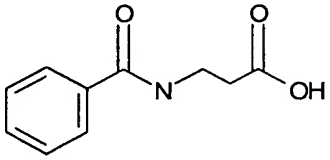
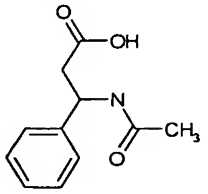
98		A	G	A	G	T	C	G	G
99		T	C	T	C	T	C	G	G
100		G	A	G	A	T	C	G	G
101		G	C	A	T	G	C	G	G
102		T	A	C	G	G	C	G	G
103		A	T	G	C	G	C	G	G
104		C	G	T	A	G	C	G	G
105		A	A	T	T	C	C	G	G
106		C	C	G	G	C	C	G	G
107		G	G	C	C	C	C	G	G
108		T	T	A	A	C	C	G	G

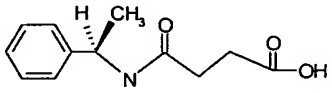
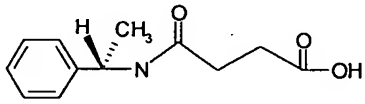
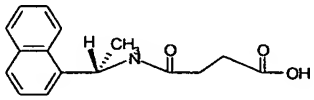
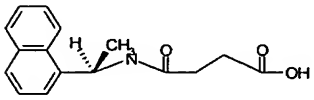
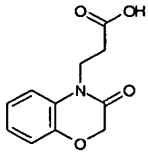
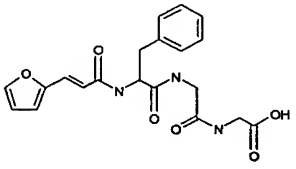
109		T	G	G	T	A	C	G	G
110		G	T	T	G	A	C	G	G
111		C	A	A	C	A	C	G	G
112		A	C	C	A	A	C	G	G
113		T	A	A	T	T	A	G	G
114		G	C	C	G	T	A	G	G
115		C	G	G	C	T	A	G	G
116		A	T	T	A	T	A	G	G

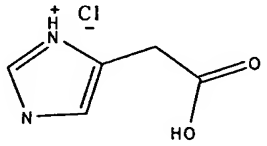
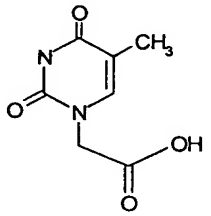
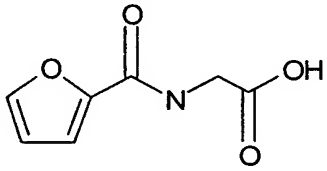
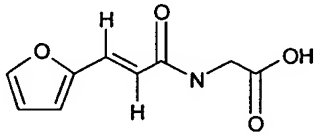
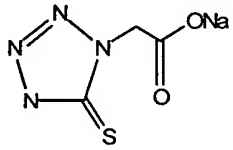
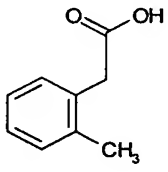
117		A	G	C	T	G	A	G	G
118		G	A	T	C	G	A	G	G
119		T	C	G	A	G	A	G	G
120		G	T	G	T	C	A	G	G
121		T	G	T	G	C	A	G	G
122		A	C	A	C	C	A	G	G
123		C	A	C	A	C	A	G	G
124		A	A	G	G	A	A	G	G

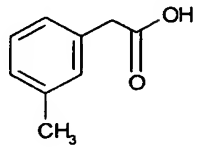
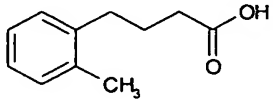
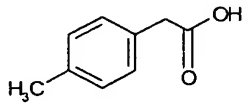
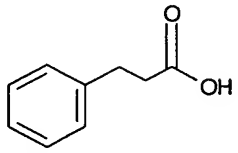
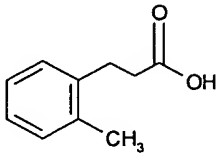
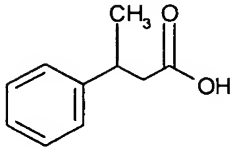
125		T	T	C	C	A	A	G	G
126		G	G	A	A	A	A	G	G
127		C	C	T	T	T	T	C	C
128		A	A	G	G	T	T	C	C
129		T	T	C	C	T	T	C	C
130		G	T	G	T	G	T	C	C
131		T	G	T	G	G	T	C	C

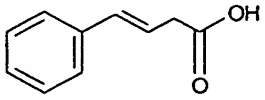
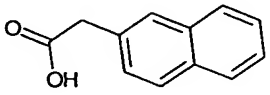
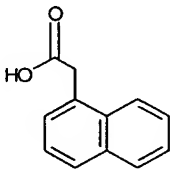
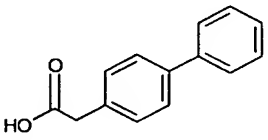
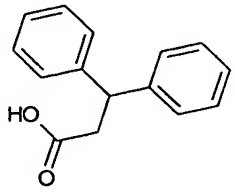
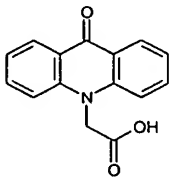
132		A	C	A	C	G	T	C	C
133		C	A	C	A	G	T	C	C
134		A	G	C	T	C	T	C	C
135		C	T	A	G	C	T	C	C
136		G	A	T	C	C	T	C	C
137		T	C	G	A	C	T	C	C

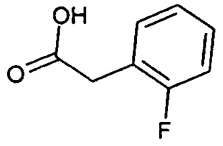
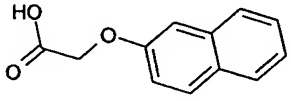
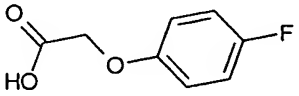
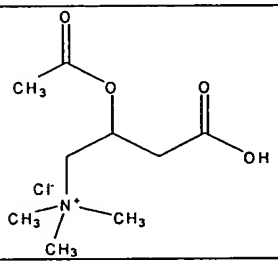
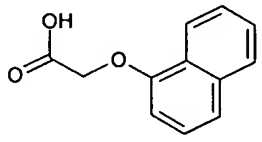
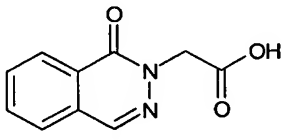
138		T	A	A	T	A	T	C	C
139		G	C	C	G	A	T	C	C
140		C	G	G	C	A	T	C	C
141		A	T	T	A	A	T	C	C
142		T	G	G	T	T	G	C	C
143		G	T	T	G	T	G	C	C

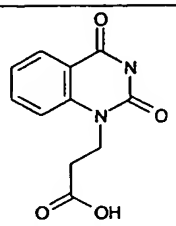
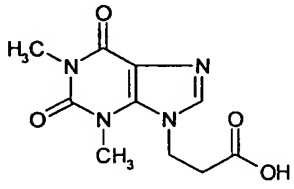
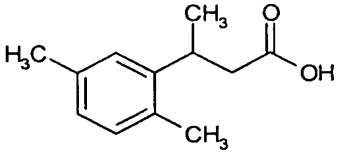
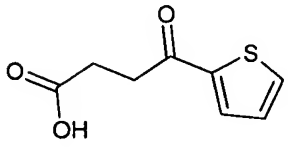
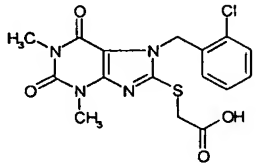
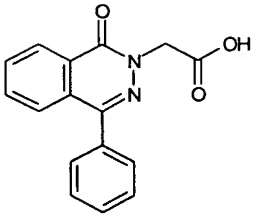
144		C	A	A	C	T	G	C	C
145		A	C	C	A	T	G	C	C
146		A	A	T	T	G	G	C	C
147		C	C	G	G	G	G	C	C
148		G	G	C	C	G	G	C	C
149		G	C	A	T	C	G	C	C

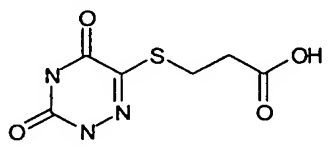
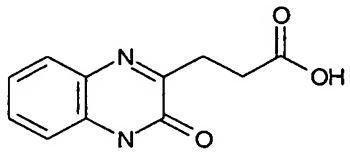
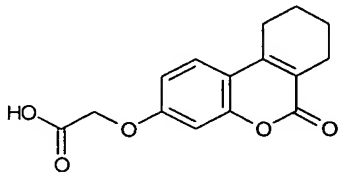
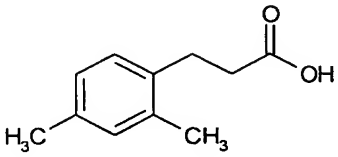
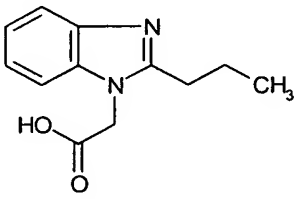
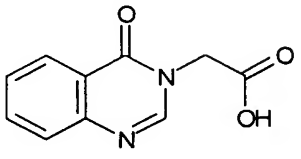
150		T	A	C	G	C	G	C	C
151		A	T	G	C	C	G	C	C
152		C	G	T	A	C	G	C	C
153		C	T	C	T	A	G	C	C
154		A	G	A	G	A	G	C	C
155		T	C	T	C	A	G	C	C

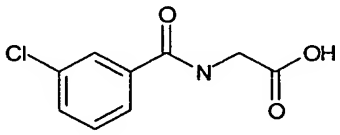
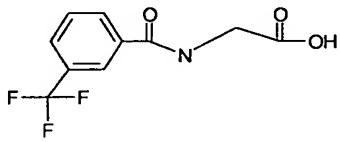
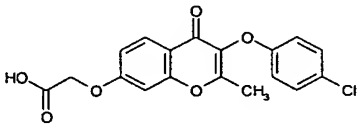
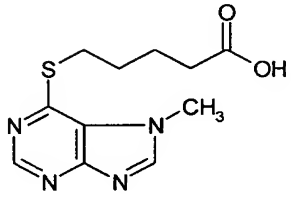
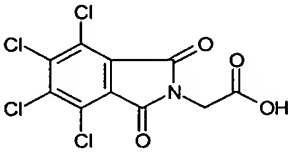
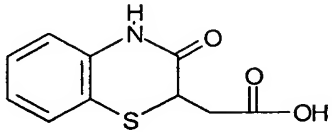
156		G	A	G	A	A	G	C	C
157		G	A	C	T	T	C	C	C
158		T	C	A	G	T	C	C	C
159		A	G	T	C	T	C	C	C
160		C	T	G	A	T	C	C	C
161		C	G	A	T	G	C	C	C

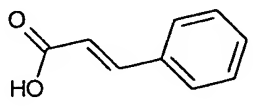
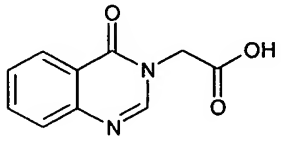
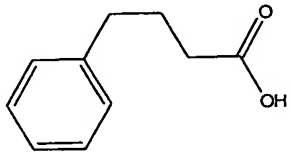
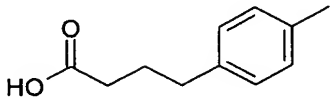
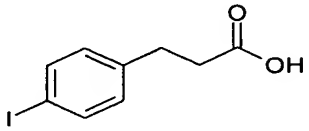
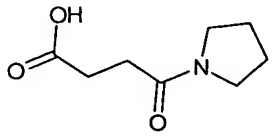
162		A	T	C	G	G	C	C	C
163		T	A	G	C	G	C	C	C
164		G	C	T	A	G	C	C	C
165		T	T	T	T	C	C	C	C
166		G	G	G	G	C	C	C	C
167		C	C	C	C	C	C	C	C

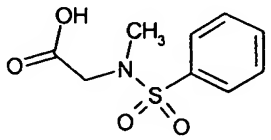
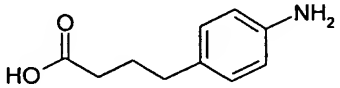
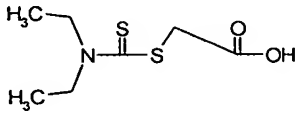
168		A	A	A	A	C	C	C	C
169		A	C	G	T	A	C	C	C
170		C	A	T	G	A	C	C	C
171		G	T	A	C	A	C	C	C
172		T	G	C	A	A	C	C	C
173		A	T	A	T	T	A	C	C

174		C	G	C	G	T	A	C	C
175		G	C	G	C	T	A	C	C
176		T	A	T	A	T	A	C	C
177		T	C	C	T	G	A	C	C
178		G	A	A	G	G	A	C	C
179		C	T	T	C	G	A	C	C

180		A	G	G	A	G	A	C	C
181		C	A	G	T	C	A	C	C
182		A	C	T	G	C	A	C	C
183		T	G	A	C	C	A	C	C
184		G	T	C	A	C	A	C	C
185		G	G	T	T	A	A	C	C

186		T	T	G	G	A	A	C	C
187		A	A	C	C	A	A	C	C
188		C	C	A	A	A	A	C	C
189		A	A	T	T	T	T	A	A
190		C	C	G	G	T	T	A	A
191		T	T	A	A	T	T	A	A

192		T	G	G	T	G	T	A	A
193		G	T	T	G	G	T	A	A
194		C	A	A	C	G	T	A	A
195		A	C	C	A	G	T	A	A
196		C	T	C	T	C	T	A	A
197		A	G	A	G	C	T	A	A

198		T	C	T	C	C	T	A	A
199		G	A	G	A	C	T	A	A
200		G	C	A	T	A	T	A	A

Preparation of the D-desthiobiotin oligonucleotide-conjugate (positive control)

The D-desthiobiotin-oligonucleotide-conjugate was synthesized and unambiguously encoded as described above. Prior Polymerase Klenow reaction, HPLC purification was performed and LC-ESI-MS showed the expected D-desthiobiotin oligonucleotide conjugated mass.

Example 2: Library Screening against streptavidin

Summary

Selections on streptavidin-sepharose were performed using experimental panning conditions essentially identical to the ones previously described by some of the inventors with a smaller single-pharmacophore DNA-encoded chemical library (Dumelin C.E., *et al.*, *Bioconjug Chem.*, **17**(2), 366-370 (2006).

Details

The library of Example 1, 300nM, was diluted 1:30 in PBS, spiked with D-desthiobiotin oligonucleotide-conjugate (final concentration 10pM) and 100µL was added to 50µl of streptavidin-sepharose slurry (GE Healthcare, cat.no 17-5113-01) or of sepharose slurry without streptavidin preincubated with PBS, 0.3mg/mL herring sperm DNA (Sigma). After incubation for 1 hour at 25°C the mixture was transferred to a SpinX column (Corning Costar Incorporated),

the supernatant removed, and the resin washed 4x400µL of PBS. At last the resin was resuspended in 100µL of water.

Identification of binding molecules

The codes of the oligonucleotide-compound conjugates were amplified before and after selection by PCR (50µL, 25 cycles of 1min at 94°C, 1min at 55°C, 40s at 72°C) either of 5µL of the resuspended resin or of 5µL of 100fM DEL4000 using primers DEL_P1_A (5'-GCCTCCCTCGCGCCATCAGGGAGCTTGTGAATTCTGG-3') and DEL_P2_B (5'-GCCTTGCCAGCCCGCTCAGGTAGTCGGATCCGACCAC-3') that additionally contain at one extremity a specific domain (19 nucleotide) required for high-throughput sequencing. The PCR product was purified on ion-exchange cartridge (Qiagen, 28104). Subsequent high-throughput sequencing was performed on a 454 Life Sciences-Roche GS FLX Sequencer platform.

For each high-throughput sequencing experiments, an average of 7116 sequences have been evaluated and plotted on 3D maps, as shown in Figure 4. This shows the results of the high-throughput sequence analysis performed on the library before selection (Figure 4a), on fractions eluted from an unmodified sepharose resin used as negative control (Figure 4b), or from streptavidin-coated sepharose (Figure 4c).

As expected, compounds in the library prior to selection were found to be represented in comparable amounts. When sequencing 11,933 individual codes from the library before selection, the average counts and the standard deviations for the 4,000 compounds were found to be 1.72 ± 1.42. Similarly, no striking enrichment was observed for selections on empty resin, even though some compounds displayed a 4-5-fold enrichment as a result of the panning procedure. By contrast, the decoding of the streptavidin selection revealed a preferential enrichment of certain classes of structurally-related compounds. In addition to desthiobiotin, which had been spiked into the library as positive control prior to selection, an enrichment of derivatives of the thioester moiety 78, of the ester moiety 49, as well as of pharmacophores 54, 73, 175 and 188 was observed. Fluorescent amide derivatives of compounds 49 and 78 had previously been found to bind to streptavidin with dissociation constants in the ~1 mM range, as assessed by fluorescence polarization assays (Dumelin, *et al.*, 2006), while 54, 73, 175 and 188 had not previously been reported as streptavidin binders.

Example 3: Resynthesis of binding molecules to assess dissociation constants

In order to assess whether the extension of the pharmacophore **49** and **78** moieties within the 4,000-membered chemical library (see Figure 4) would contribute to an increased affinity

towards streptavidin, the dissociation constants of the most relevant compounds were measured, following conjugation to fluorescein.

Synthesis

In a on-resin peptide synthesis syringe with 50mg (46µmol) of O-bis-(aminoethyl)ethylene glycol trityl resin (Novabiochem, cat.no 01-64-0235) were introduced in dry DMF solution the appropriate fmoc-protected aminoacid (100µmol, 1mL), HBTU (Aldrich, 200µmol, 1mL), DIEA (Fluka, 400µmol, 0.5mL). After overnight incubation at room temperature, the resin was washed 6x2mL dry DMF and the fmoc moiety removed by addition of 1mL piperidine (Fluka) 50% dry DMF for 1 hour at room temperature. Following washing 6x2mL dry DMF, the corresponding carboxylic acid (100µmol, 1mL DMF) was introduced and a further amide bond formation reaction performed as described above. The resulting product was cleaved treating the resin with 10x2mL TFA 1% in CH_2Cl_2. The DCM fractions were quenched in 5mL $NaHCO_3$ aq_{sat} and the water phase back extracted 2x5mL CH_2Cl_2. All the organic phases collected were washed with water until pH 7.0, dried on Na_2SO_4 and concentered *in vacuo*. The crude product was treated in the dark overnight at room temperature with 2 equivalents of fluorescein isothiocyanate (FITC, Aldrich) in 800µL of DMF and 200µL $NaHCO_3$ aq_{sat}. Following HPLC purification on an XTerra Prep RP_{18} column (5µM, 10x150mm) using a linear gradient from 10 % to 100% MeCN 0.1% TFA, the desired fractions were collected and lyophilized. ESI-MS analysis confirmed the mass of the expected FITC conjugated products. **02-78** ($C_{45}H_{49}BrN_4O_{10}S_2$) expected: 949.93 measured: 951.31 [$M+H^+$]; **07-78** ($C_{50}H_{59}N_5O_{12}S_2$) expected: 985.36 measured: 986.37 [$M+H^+$]; **15-78** ($C_{44}H_{49}N_5O_{12}S_3$) expected: 935.25 measured: 936.25 [$M+H^+$]; **02-107** ($C_{47}H_{47}BrN_4O_9S$) expected: 923.87 measured: 925.12 [$M+H^+$]; **13-40** ($C_{46}H_{49}N_5O_{14}S$) expected: 927.30 measured: 928.42 [$M+H^+$]; **11-78** ($C_{49}H_{58}N_4O_{13}S_2$) expected: 974.34 measured: 975.41; **17-49** ($C_{45}H_{49}IN_4O_{11}S$) expected: 980.22 measured: 981.29 [$M+H^+$]; **17-78** ($C_{45}H_{49}IN_4O_{10}S_2$) expected: 996.19 measured: 997.26; **16-78** ($C_{43}H_{48}N_4O_{10}S_3$) expected: 876.25 measured: 877.33; **15-117** ($C_{45}H_{45}N_5O_{12}S_2$) expected: 911.25 measured: 912.33; **02-49** ($C_{45}H_{49}BrN_4O_{11}S$) expected: 932.23; measured: 933.32 [$M+H^+$].

Affinity measurements

Fluorescein-compound conjugates (500nM) were incubated with increasing amounts of streptavidin (BIOSPA, cat.no S002-6) in PBS, 5% DMSO, for 1 hour at 25°C. The fluorescent polarization was determined with a TECAN Polarion instrument by excitation at 485nm and measurement at 535nm (e = 72.000 $M^{-1}cm^{-1}$).

Results

As expected, compound **02-78**, which displayed the strongest enrichment following streptavidin selection, revealed the lowest dissociation constant (Kd = 385 nM). Replacement of the p-bromophenyl moiety **02** with compound **07** or with the sulfonamide **15** led to a progressive loss of enrichment in the selection, which was paralleled by a loss of binding affinities (Kd = 11 μM and 78 μM, respectively). The p-bromophenyl moiety **02**, which contributes to the binding affinity of compound **02-78**, was not able to yield high streptavidin binding affinities when used in the absence of pharmacophore **78**, as revealed by the low enrichment profiles of **02**-containing compoundsand by the large dissociation constants of compounds in the series (e.g., Kd = 50 μM for **02-107**).

Compound	K_D [μM]
02-78	0.385
07-78	11
15-78	78
02-107	50

Example 4: Library screening against IgG

Summary

In a similar manner to Example 2, selections against IgG-sepharose were performed.

Details

Polyclonal human IgG coating of sepharose beads

100mg CNBr-activated sepharose (GE Healthcare, Piscataway, NJ) was swollen in 1 mM HCl, washed, and mixed in separate tubes with 2.5 mg/ml polyclonal human IgG (Sigma-Aldrich-Fluka, Buchs, Switzerland) dissolved in 0.1 M Tris-Cl, 0.5 M NaCl, pH 8.3. After 4 hour incubation at 4°C, the slurry was repeatedly washed and stored in 0.1 M NaAc, 0.5 M NaCl, pH 4 at 4°C.

Library Screening

The library of example 1 (total oligonucleotide conjugate concentration 300nM) was diluted 1:30 in PBS. 100μL of the library was either added to 50μl IgG-sepharose slurry or to sepharose slurry without streptavidin. Both resins were preincubated with PBS, 0.3mg/mL herring sperm DNA (Sigma). After incubation for 1 hour at 25°C the mixture was transferred to a SpinX column (Corning Costar Incorporated), the supernatant was removed, and the resin washed 4x with 400μL PBS. After washing, the resin was resuspended in 100μL water.

Identification of binding molecules

The codes of the oligonucleotide-compound conjugates were amplified by PCR (50µL, 25 cycles of 1 minute at 94°C, 1 minute at 55°C, 40 seconds at 72°C) with either 5µL of 100fM DEL4000 library before selection as template, or 5µL of each resuspended resin after selection as template. The PCR primers DEL_P1_A (5'-**GCC TCC CTC GCG CCA TCA G**GG AGC TTG TGA ATT CTG G-3') and DEL_P2_B (5'-**GCC TTG CCA GCC CGC TCA G**GT AGT CGG ATC CGA CCA C-3') additionally contain at one extremity a 19 nucleotide domain (underlined) required for high-throughput sequencing with the 454 Genome Sequencer system. The PCR products were purified on ion-exchange cartridges (Qiagen, 28104). Subsequent high-throughput sequencing was performed on a 454 Life Sciences-Roche GS Flx Sequencer platform.

For each high-throughput sequencing experiments 39.092 sequences have been evaluated and plotted on 3D maps, as shown in Figure 5. This shows the results of the high-throughput sequence analysis performed on the library before selection, on fractions eluted from an unmodified sepharose beads used as negative control, or from IgG-coated sepharose beads.

Results

The decoding of the polyclonal IgG selection showed a preferential enrichment of certain classes of structurally-related compounds. Typically an enrichment of the derivatives of compound 40 as well as of the pharmacophore 69 was observed. Additionally the derivatives of the bromide 02 and of thiophene 16 revealed an exceptional enrichement in combination both with the moiety 40 and the compound 69.

Example 5: Resynthesis of binding molecules to assess dissociation constants

In order to assess the dissociation constant of the most relevant compounds selected (see Figure 5), fluorescent polarization was measured, following resynthesis of the compounds and conjugation to fluorescein.

Details

Synthesis of the binding molecules as fluorescein conjugates

In a polypropylene syringe, 50mg (46µmol) of O-bis-(aminoethyl)ethylene glycol trityl resin (Novabiochem, cat.no 01-64-0235) was suspended in a mixture of the appropriate fmoc-protected amino acid (100µmol , 1mL), HBTU (Aldrich, 200µmol , 1mL), and DIEA (Fluka, 400µmol , 0.5mL) in dry DMF. After overnight incubation at 25°C, the resin was washed 6x with 2mL dry DMF and the fmoc moiety was removed by addition of 1mL piperidine (50% in dry

DMF) for 1 hour at 25°C. After washing 6x with 2mL dry DMF, the corresponding carboxylic acid (100µmol, 1mL DMF) was added and a further amide bond formation reaction was performed as described above. The resulting product was cleaved by treating the resin with 10x with 2mL TFA (1% in CH_2Cl_2). The dichloromethylene fractions were quenched in 5mL $NaHCO_3$ aq_{sat} and the water phase was back extracted 2x with 5mL CH_2Cl_2. The pooled organic phases were washed 3x with water, dried on Na_2SO_4 and concentrated *in vacuo*. The crude product was reacted with 2 equivalents of fluorescein isothiocyanate (800µL of DMF) and 200µL $NaHCO_3$ aq_{sat} in the dark overnight at 25°C. Following HPLC purification on an XTerra Prep RP_{18} column (5µM, 10x150mm) using a linear gradient from 10% to 100% MeCN 0.1% TFA, the desired fractions were collected and lyophilized. ESI-MS analysis confirmed the mass of the expected FITC conjugated products. **02-40** ($C_{29}H_{41}BrN_4O_9$) expected: 668.21 measured: 669.37 [$M+H^+$]; **16-40** ($C_{27}H_{40}N_4O_9S$) expected: 596.25 measured: 597.12 [$M+H^+$].

Affinity measurements

Fluorescein-compound conjugates (500 nM) were incubated with increasing amounts of polyclonal human IgG (Sigma-Aldrich-Fluka, Buchs, Switzerland) in PBS, 5% DMSO, for 1 hour at 25°C. The fluorescent polarization was determined with a TECAN Polarion instrument by excitation at 485nm and measuring emission at 535nm (ε = 72000 $M^{-1}cm^{-1}$).

The apparent association constant revealed was 200 µM.

Example 6: Synthesis of IgG-binding resin

In order to asses the performance of the compounds selected in affinity chromaphy purification of a human polyclonal IgG sample, the compounds selected were immobilized on a suitable affinity chromatograpy resin support and applied both in the affinity chromatograpy of a pure sample of polyclonal human IgG Cy5 labelled and in the purification of a crude sample of a CHO (Chinese Hamster Ovary) cells supernatant spiked with plyclonal human IgG Cy5 labelled.

Synthesis of the binding molecules on affinity chromatography resin

In a polypropylene syringe, 50mg (46µmol) of O-bis-(aminoethyl)ethylene glycol trityl resin (Novabiochem, cat.no 01-64-0235) was suspended in a mixture of the appropriate fmoc-protected amino acid (100µmol, 1mL), HBTU (Aldrich, 200µmol, 1mL), and DIEA (Fluka, 400µmol, 0.5mL) in dry DMF. After overnight incubation at 25°C, the resin was washed 6x with 2mL dry DMF and the fmoc moiety was removed by addition of 1mL piperidine (50% in dry DMF) for 1h at 25°C. After washing 6x with 2mL dry DMF, the corresponding carboxylic acid (100µmol, 1mL DMF) was added and a further amide bond formation reaction was performed

as described above. The resulting product was cleaved by treating the resin with 10x with 2mL TFA (1% in CH_2Cl_2). The dichloromethylene fractions were quenched in 5mL $NaHCO_3$ aq_{sat} and the water phase was back extracted 2x with 5mL CH_2Cl_2. The pooled organic phases were washed 3x with water, dried on Na_2SO_4 and concentrated *in vacuo*. The crude product was reacted with 2 equivalents of fluorescein isothiocyanate (800µL of DMF) and 200µL $NaHCO_3$ aq_{sat} in the dark overnight at 25°C. Following HPLC purification on an XTerra Prep RP_{18} column (5µM, 10x150mm) using a linear gradient from 10% to 100% MeCN 0.1% TFA, the desired fractions were collected and lyophilized. ESI-MS analysis confirmed the mass of the expected products. 200mg CNBr-activated sepharose (GE Healthcare, Piscataway, NJ) was swollen in 1 mM HCl, washed, and mixed in separate tubes with 15µmol of the compounds dissolved in 2mL 0.1M $NaHCO_{3aq}$, 10% DMF. After 4 hours incubation at 25°C, the slurry was repeatedly washed and stored in PBS at 4°C.

Polyclonal human IgG Cy5 labeling

Polyclonal human IgG (Sigma-Aldrich-Fluka, Buchs, Switzerland) was labelled with Cy5 Mono-reactive kit (Amersham, cat.no PA25001) according to the protocol of the provider and purified over a PD10 column (GE Healthcare, cat.no 17-0851-01) as described by the supplier.

Affinity chromatography on IgG binding resin (Figure 6a)

70mg of L2C2C40 or L2C16C40 resin were loaded on a chromatography cartridge (Glen Research, cat.no 20-0030-00) and washed 3x with 1mL PBS before loading Cy5 labelled polyclonal human IgG solution (216µL, 4.64µM in PBS). The flow-through of the washing steps (washing 1x with 1mL PBS; 1x with 1mL 500mM NaCl, 0.5mM EDTA; 1x with 1mL 100 mM NaCl, 0.1% Tween 20, 0.5 mM EDTA) and the elutate (elution 3x with 200µL aqueous triethylamine (TEA) 100mM) were collected and eventually concentrated back to a final volume of 200µL by centrifugation in a Vivaspin 500 tube (Vivascience, cat.no VS0101, cut-off 10.000 MW). The samples were then analyzed by gel electrophoresis on a NuPAGE 4-12% Bis-Tris Gel (Invitrogen, cat.no NP0321) using MOPS SDS as running buffer and stained with Coomassie Blue. The results are shown in Figure 6a, wherein:

M = Marker Rainbow Full range

In = Polyclonal Human IgGCy5 2 nmol in 216 µL PBS

W1 = 1 mL PBS

W2 = 1 mL 500 mM NaCl, 0.5 mM EDTA

W3 = 1 mL 100 mM NaCl, 0.1% Tween 20, 0.5 mM EDTA

E1 = E2 = E3 = 200 µL 0.1 M TEA

Affinity chromatography of crude a CHO (Chinese Hamster Ovary) cells supernatant spiked with human IgG Cy5 labelled on IgG binding resin (Figure 6b)

70mg of L2C2C40 or L2C16C40 resin were loaded on a chromatography cartridge (Glen Research, cat.no 20-0030-00) and washed 3x with 1mL PBS before loading a crude sample of CHO (Chinese Hamster Ovary) cells supernatant (60 µL) spiked with human IgG Cy5 labelled (20 µL, 9.68 µM). The flow-through, the washing steps (washing 1x with 10 mL PBS; 1x with 10 mL 500 mM NaCl, 0.5 mM EDTA; 1x with 10 mL 100 mM NaCl, 0.1% Tween 20, 0.5 mM EDTA) and the elutate (elution 3x with 200 µL aqueous triethylamine 100 mM) were collected and eventually concentrated back to a final volume of 100µL by centrifugation in a Vivaspin 500 tube (Vivascience, cat.no VS0101, cut-off 10.000 MW). The samples were then analyzed by gel electrophoresis on a NuPAGE 4-12% Bis-Tris Gel (Invitrogen, cat.no NP0321) using MOPS SDS as running buffer and stained with Coomassie Blue. Cy5 activity was detected by a Diana III Chemiluminescence Detection System (Raytest) by excitation at 675 nm and measuring emission at 694 nm (ε = 250,000 $M^{-1}cm^{-1}$). The results are shown in Figure 6b, wherein:

M = Marker Rainbow Full Range
In = 60 µL supernatant + 20 IgGCy5 9.68 mM
W = 10 mL PBS + 10 mL 500 mM NaCl, 0.5 mM EDTA +
10 mL 100 mM NaCl, 0.1% Tween 20, 0.5 mM EDTA
E1 = E2 = E3 = 200 µL 0.1 M TEA
Ctrl(+) = Polyclonal Human IgG

Results

As it is shown in Figure 6, the human IgG Cy5 capture by the resin and subsequently elution is in both the experiments quantitative (Figure 6a and 6b). Additionally Figure 6b shows the high selectivity of the resin for the capture of human IgG Cy5 after extensive washing and elution.

Example 7: Stepwise encoding

Introduction

Figure 7 shows three different experimental schemes, which could be conceived for the construction and encoding of a large library. The first scheme features the stepwise addition of groups of chemical moieties onto an initial scaffold, using suitable orthogonal chemical reactions and/or protecting strategies, followed by the sequential addition of the corresponding DNA codes by an iterative ligation procedure. This scheme, which corresponds broadly to known methods, is conceptually simple and can be implemented experimentally, but requires 2 pre-annealed oligonucleotides for each encoding event (i.e., 200 + 200 + 200 oligonucleotides for a library containing 100 x 100 x 100 chemical groups; Figure 7a). The second synthetic and

encoding strategy represents an embodiment of the first aspect of the invention. This method uses Klenow polymerization, which would require the lowest number of oligonucleotides for library encoding (100 + 100 + 100 oligonucleotides for a library containing 100 x 100 x 100 chemical groups; Figure 7b). Finally, an embodiment of the second aspect of the invention is shown/ Here, the double-stranded DNA fragment generated by Klenow filling is followed by a digestion with a non-palindromic cutter and by a subsequent ligation step with a complementary double-stranded DNA fragment (Figure 7c)

The feasibility of the experimental procedures is demonstrated below by gel-electrophoretic analysis, which monitored the stepwise assembly of DNA-fragments of suitable size, and by DNA sequencing.

General methods

Gel electrophoresis was performed either using 15% Tris-Borate-EDTA-Urea denaturing polyacrylamide gels (TBE-Urea, Invitrogen, cat.no EC68852) or 20% Tris-Borate-EDTA native polyacrylamide gels (TBE, Invitrogen, cat.no EC63152) and stained with SYBRgreenII (Sigma, S9305). DNA Ethanol precipitation of DNA was performed by adding 1/10 volumes of 3M AcOH/AcONa buffer pH 4.7, and 3 volumes of ethanol relative to the volume of the DNA sample. After 2h incubation at -23°C the mixture was centrifuged in a table-top centrifuge for 40min (16.000*g*) at 4°C, the supernatant removed and the pellet washed with 300 µL ice-cold ethanol 90%. After a further 20min centrifugation (16.000*g*) at 4°C, the pellet was dried and redissolved in water.

*Comparative example: Stepwise encoding by Ligation (Method **a** Underlined sequences represent coding sequences)*

Hybridization of 3 pairs (A, B, C) of oligonucleotides (A: 5'-CAT GGA ATT CGC TCA CTC CGA CTA GAG G-3' and 5'-(Phosphate)-CGT ACC TCT AGT CGG AGT GAG CGA ATT CCA TG-3'; B: 5'-(Phosphate)-TAC GTG AGC TTG ACC TGG TGA G-3' and 5'- (Phosphate)-GCT TCT CAC CAG GTC AAG CTC A-3'; C: 5'-(Phosphate)-AAG CAC GTT CGC TGG ATC CTC AAC TGT G-3' and 5'-CAC AGT TGA GGA TCC AGC GAA CGT-3', IBA) was carried out by mixing the oligonucleotides at a concentration of 1.25 µM per oligonucleotide in 1x ligase buffer (40 mM Tris-HCl, 10 mM MgCl2, 10 mM DTT, 0.5 mM ATP, pH 7.8) and incubating the mixtures for 10 minutes at 50°C. Subsequently the ligations were performed mixing 10 µl of hybridized oligonucleotide pairs A and B with 10 µl of 1x ligase buffer and 1 µL of T4 ligase (Roche Applied Science, Basel, Switzerland), and incubated at room temperature for 2 hours. The ligation product was purified using a Qiagen Nucleotide Removal Kit (Qiagen, cat.no 28306), and eluted

with 50 µl of 10 mM Tris-HCl pH 8.0. 18 µl of the eluate was mixed with 10 µl of hybridized oligonucleotide pair C (which was present in excess), 2 µl of 10x ligase buffer, and 1 µl of T4 ligase, and incubated for 2 hours at room temperature. Aliquots of the two starting oligonucleotides, and the different ligation products were subjected to electrophoresis on a 20% TBE gel.

*Stepwise encoding by Klenow Polymerase (Method **b** Underlined sequences represent coding sequences)*

To a reaction volume of 50µL, reagents were added to the respective final concentrations: a 42mer 5'-amino-C12-DNA-oligonucleotide (5'-GGA GCT TGT GAA TTC TGG ATC TTA GGA CGT GTG TGA ATT GTC-3', IBA), 2µM, 42mer 3'-C6-biotinylated-oligonucleotide (5'-GTA GTC GGA TCC GAC CAC GTT CCT GAC AAT TCA CAC ACG TCC-3', IBA), 3µM, Klenow buffer (NEB, cat.no B7002S), dNTPs (Roche, cat.no 11969064001), 0.5mM, Klenow Polymerase enzyme (NEB, cat.no M0210L), 5 units. The Klenow polymerization reaction was incubated at 37°C for 1h, purified on ion-exchange cartridge (Qiagen, cat.no 28306) and eluted in 100µL of 4M urea. After incubating at 94°C for 2min, 50µl of streptavidin-sepharose slurry (GE Healthcare, cat.no 17-5113-01) were added and the slurry was incubated for 1h at 4°C. The streptavidin sepharose resin and the supernatant were separated by centrifugation in a SpinX column (Corning Costar Incorporated). The DNA in the supernatant was ethanol precipitated as described above. The resulting singele-stranded oligonucleotide was mixed with a 42mer unmodified DNA oligonucleotide (5'-GTC GTA TCG CCA TGG TCC AAC ATC GTA GTC GGA GAG GAC CAC-3') and a Klenow polymerization reaction was performed as described above. Aliquots of the three starting oligonucleotides, and the different Klenow products were applied on a 15% TBE-Urea gel.

*Stepwise encoding by "Hybrid Klenow-Ligation" (Method **c** Underlined sequences represent coding sequences))*

To a reaction volume of 50µL, reagents were added to the respective final concentrations: a 42mer 5'-amino-C12-DNA-oligonucleotide (5'-GGA GCT TGT GAA TTC TGG ATC TTA GGA CGT GTG TGA ATT GTC-3', IBA), 2µM, a 42mer 5'-C6-biotinylated-oligonucleotide containing the non-palindromic BssSI restriction site (in boldface type) (5'-GTA GTC GGA **CAC GAG** TAC TGG TAA TCG ACA ATT CAC ACA CGT CC–3', IBA), 3µM, klenow buffer (NEB, cat.no B7002S), dNTPs (Roche, cat.no 11969064001), 0.5mM, and Klenow Polymerase enzyme (NEB, cat.no M0210L), 5 units. After incubation at 37°C for 1h, the reaction mixture was purified on ion-exchange cartridge (Qiagen, cat.no 28306) and eluted in 25 µL of water. 8 units of BssSI enzyme were added to the purified Klenow product in 50µL of BssSI restriction buffer (NEB,

cat.no B7003S) . The restriction cutting reaction was carried out at 37°C for 1.5h. 50µL of streptavidin-sepharose slurry (GE Healthcare, cat.no 17-5113-01) was added and the slurry was incubated for 30min at 4°C. After SpinX column centrifugation (Corning Costar Incorporated), the supernatant was collected and purified on ion-exchange cartridge (Qiagen, cat.no 28306) and eluted in 25 µL of water. Subsequently we added the following reagents to the final volume of 50µL: preincubated mixture 1:1 of hybridized oligonucleotides (27mer 5`-pyrophosphate-TCG TGA AAT TTG CTA GGA TCC ATA TTG–3` and 23mer 5`-CAA TAT GGA TCC TAG CAA ATT TC–3`, IBA), 3µM, T4 ligase buffer (Roche Applied Science, Basel, Switzerland) and T4 ligase (Roche Applied Science, Basel, Switzerland), 4 units. The ligation was performed overnight at 16°C then purified on ion-exchange cartridge (Qiagen, cat.no 28306). Aliquots of the starting oligonucleotides, and the different Klenow, restriction and ligation products were analyzed on a 15% TBE-Urea gel.

Results

The three methods described above allow to succesfully complete the stepwise encoding (see gels Figure 7). Compared with the ligation method (method a, Figure 7), the Klenow Polymerase and the hybrid Klenow-ligation methods allow the same stepwise encoding minimizing the number of oligonucleotides required (method b and c, Figure 7).

Example 8

Introduction

Figure 8a shows a further experimental scheme, which could be conceived for the construction and encoding of a large library according to the third aspect of the invention.

The feasibility of the experimental procedures is demonstrated below by gel-electrophoretic analysis, which monitored the stepwise assembly of DNA-fragments of suitable size, and by DNA sequencing. The analysis was carried out using the same technique as described in Example 7.

Stepwise encoding by "Klenow-Ligation" (Underlined sequences represent coding sequences)

To a reaction volume of 50µL, reagents were added to the respective final concentrations: a 42mer 5'-amino-C12-DNA-oligonucleotide (5'-GGA GCT TGT GAA TTC TGG ATC TTA GGA CGT GTG TGA ATT GTC-3', IBA), 320nM, a 44mer oligonucleotide containing the restriction site BamHI (boldface type) (5'-GTA GTC **GGA TCC** GAC CAC GCA TAT AAG ACA ATT CAC ACA CGT CC-3',IBA), 600nM, klenow buffer (NEB, cat.no B7002S), dNTPs (Roche, cat.no 11969064001), 0.5mM, and Klenow Polymerase enzyme (NEB, cat.no M0210L), 5 units. After

incubation at 37°C for 1 hour, the reaction mixture was purified on ion exchange cartridge (Qiagen, cat.no 28306) and eluted in 33 µL of water. 10 units of BamHI enzyme were added to the purified Klenow product (100nM) in 50µL of BamHI restriction buffer (NEB, cat.no B7003S) containing 0.1mg/mL BSA. The restriction cutting reaction was carried out at 37°C for 12 hours and purified on ion-exchange cartridge (Qiagen, cat.no 28306) and eluted in 50 µL of water. Subsequently the following reagents were added to a reaction volume of 25µL: purified restriction product (20nM), preincubated mixture 1:1 of hybridized oligonucleotides (43mer 5`-pyrophosphate-GAT CCG CTG CCC CGC CAA AAA AAA GAT CTG ATG GCG CGA GGG A -3` and 12mer 5`- GGC GGG GCA GCG `, Sigma), 100nM, T4 ligase buffer (NEB) and 1 µL T4 ligase (NEB, cat.no M0202L). The ligation was performed overnight at 16°C and then purified on ion exchange cartridge (Qiagen, cat.no28306). The final product was PCR amplified using the primers 5`-GGA GCT TGT GAA TTC TGG-3`and 5`-GCC TCC CTC GCG CCA TCA GAT C-3` and analysed by subcloning and sequencing.

Aliquots of the starting oligonucleotides, and the different Klenow, restriction and ligation products were analyzed on a 20% TBE gel (Figure 8b), wherein the reference numerals relate to Figure 8a.

Claims

1. A method of preparing a DNA-encoded chemical library comprising members which comprise a chemical moiety, a linking moiety and a DNA moiety, comprising for each member the steps of:

(I) coupling a initial building block to a initial coding single strand oligomer via a linking moiety to form a initial conjugate, the initial coding single strand oligomer comprising a first primer region, an initial coding region and a first annealing region;

(II) optionally coupling a middle building block and middle coding single strand oligomer to said initial conjugate to form a middle conjugate, the coupling take place in either order, wherein:

(a) the middle building block is coupled to the residue of the initial building block; and

(b) the middle coding single strand oligomer comprises a middle coding region and second annealing region, and is coupled by:

(i) annealing a complementary single strand oligomer which comprises a chemical modifier, a complementary first annealing region, a complementary middle coding region and a complementary second annealing region by interaction between the first annealing region and the complementary first annealing region;

(ii) treating the conjugate formed with a DNA polymerase to elongate the initial coding single strand oligomer to be complementary to the complementary middle coding region and the complementary second annealing region;

(iii) removing the complementary single strand oligomer by denaturing the DNA and capturing the complementary single strand oligomer via the chemical modifier;

(III) coupling a final building block and final coding single strand oligomer to the initial or middle conjugate as appropriate to form a final conjugate, the coupling take place in either order, wherein:

(a) the final building block is coupled to the residue of the initial building block, or may be additionally or alternatively be coupled to the residue of the middle building block (if present); and

(b) the final coding single strand oligomer comprises a final coding region and a second primer region, and is coupled by:

(i) annealing a complementary single strand oligomer which comprises a complementary first or second annealing region as appropriate, a complementary final coding region and a complementary second primer region, by interaction between the first or second annealing region and the complementary first or second annealing region, as appropriate;

(ii) treating the conjugate formed with a DNA polymerase to elongate the initial coding

single strand oligomer to be complementary to the complementary final coding region and the complementary second primer region, and to elongate the complementary coding strand to be complementary to the initial coding single strand oligomer.

2. A method of preparing a DNA-encoded chemical library comprising a chemical moiety, a linking moiety and a DNA moiety, comprising for each member the steps of:

(I) coupling a initial building block to a initial coding single strand oligomer via a linking moiety to form a initial conjugate, the initial coding single strand oligomer comprising a primer region, an initial coding region and an annealing region;

(II) coupling a middle building block and middle coding single strand oligomer to said initial conjugate to form a middle conjugate, the coupling take place in either order, wherein:

(a) the middle building block is coupled to the residue of the initial building block; and

(b) the middle coding single strand oligomer comprises a middle coding region and a restriction region, and is coupled by:

(i) annealing a complementary single strand oligomer which comprises a complementary annealing region, a complementary middle coding region, a complementary restriction region and a chemical modifier, by interaction between the annealing region and the complementary annealing region;

(ii) treating the conjugate formed with a DNA polymerase to elongate the initial coding single strand oligomer to be complementary to the a complementary middle coding region and the complementary restriction region and to elongate the complementary single strand oligomer to be complementary to the initial primer region and initial coding region; and

(iii) treating the conjugate with a non-palindromic restriction enzyme which acts on the restriction region to leave a middle conjugate, and capturing the restriction product via the chemical modifier;

(III) coupling a final building block and final coding single strand oligomer to the middle conjugate as appropriate to form a final conjugate, the coupling take place in either order, wherein:

(a) the final building block is coupled to the residue of the initial building block and/or to the residue of the middle building block; and

(b) the final coding single strand oligomer comprising a final coding region and a second primer region, and is coupled by ligating a terminal double-stranded portion comprising a ligation region that matches the remainder of the restriction region site of the middle conjugate, and a final coding region and second primer region, as well as the complementary regions in the complementary strand.

3. A method of preparing a DNA-encoded chemical library comprising members which comprise a chemical moiety, a linking moiety and a DNA moiety, comprising for each member the steps of:

(I) coupling a initial building block to a initial coding single strand oligomer via a linking moiety to form a initial conjugate, the initial coding single strand oligomer comprising a first primer region, an initial coding region and a first annealing region;

(II) coupling a middle building block and middle coding single strand oligomer to said initial conjugate to form a middle conjugate, the coupling take place in either order, wherein:

(a) the middle building block is coupled to the residue of the initial building block; and

(b) the middle coding single strand oligomer comprises a middle coding region and a restriction region, and is coupled by:

(i) annealing a complementary single strand oligomer which comprises a complementary first annealing region, a complementary middle coding region and a complementary restriction region by interaction between the first annealing region and the complementary first annealing region;

(ii) treating the conjugate formed with a DNA polymerase to elongate the initial coding single strand oligomer to be complementary to the complementary middle coding region and the complementary restriction region;

(iii) treating the conjugate with a restriction enzyme which acts on the restriction region to leave a middle conjugate, and removing the restriction product with a chromatographic method;

(III) coupling a final building block and final coding partially double stranded oligomer to the middle conjugate to form a final conjugate, the coupling take place in either order, wherein:

(a) the final building block is coupled to the residue of the initial building block, and additionally or alternatively be coupled to the residue of the middle building block; and

(b) the final coding partially double stranded oligomer comprises a final coding region, a second primer region, and a double stranded ligation region that matches the remainder of the restriction region site of the middle conjugate, and is coupled by ligation using an excess of the final coding partially double stranded oligomer.

4. A method according to any one of claims 1 to 3, wherein the individual method steps are carried out at about the same time for each library member.

5. A method according to any one of claims 1 to 4, wherein the relative amounts of the individual members within the library varies from about 0.2 equivalents to about 10 equivalents.

6. A method according to any one of claims 1 to 5, wherein the building blocks are selected

from compounds having one or more of the following moieties: amine, carboxylic acid, ester, acid anhydride, acid halide, a double bond conjugated to an electron withdrawing group, thiol, alcohol, enolate, azide, alkyne, hydroxy, halogen, OTf, ONf, OTs, ketone and boron containing moiety.

7. A method according to claim 6, wherein the initial building block comprises a protected amine group which is deprotected before being coupled to a middle or final building block as appropriate.

8. A method according to any one of claims 1 to 7, wherein the linker moiety has a chain length of 1 to 20 atoms.

9. A method according to any one of claims 1 to 8, wherein the first and second primer moieties comprise between 16 and 28 nucleotides.

10. A method according to any one of claims 1 to 9, wherein the annealing regions comprise between 10 and 28 nucleotides.

11. A method according to any one of claims 1 to 10, wherein the chemical modifier, if present, is a biotin or imminobiotin moiety.

12. A DNA-encoded chemical library synthesised by a method according to any one of claims 1 to 11.

13. A DNA-encoded chemical library comprising members which comprise a chemical moiety, a linking moiety and a DNA moiety, wherein the chemical moiety is the reaction product of an initial building block and a final building block, the initial building blocks being selected from table 1 and the final building blocks being selected from table 2 where the library comprises members representing the reaction product of at least three initial building blocks and at least 5 final building blocks, and the DNA moiety comprises a first primer region, an initial coding region, a first annealing region, a final coding region and a second primer region,

14. A DNA-encoded chemical library according to claim 13, wherein the linking moiety is a 5'-amino modifier C12.

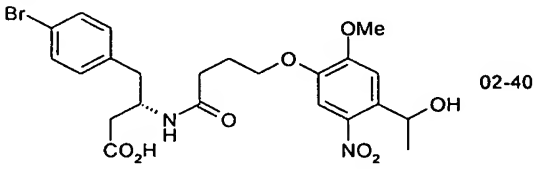
15. A DNA-encoded chemical library according to either claim 13 or claim 14, wherein the

initial coding region comprises 6 base pairs and the final coding region comprises 8 base pairs.

16 A DNA-encoded chemical library according to any one of claims 13 to 15, wherein the first annealing region comprises 18 base pairs.

17. A DNA-encoded chemical library according to any one of claims 13 to 16, wherein the first and second primer regions are usuable with the PCR primers DEL_P1_A and DEL_P2_B respectively.

18. A compound selected from:



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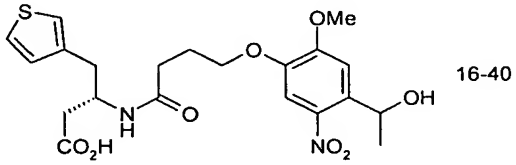

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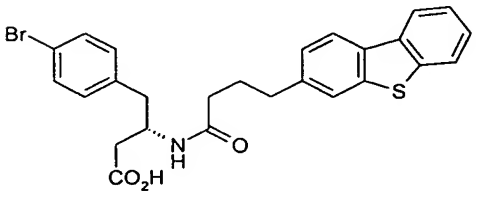

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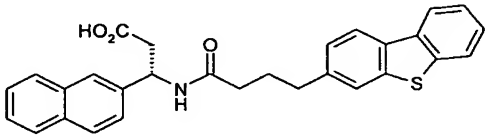
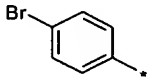
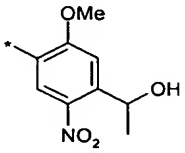


18-69

19. Use of a compound according to claim 18 as an affinity ligand in an affinity chromatography resin for IgG.

20. An affinity chromatography resin for IgG comprising as the affinity ligand a moiety comprising a pair of constituent moieties being:

a) 



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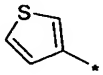
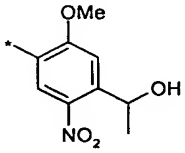
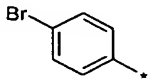
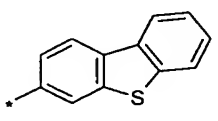
b) 

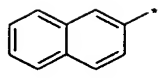
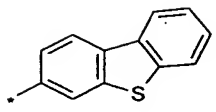


;

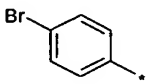
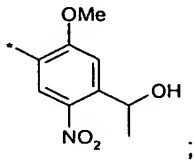
c) 

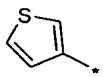
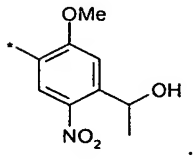


; or

d)  

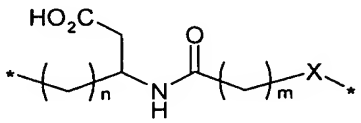
21. An affinity chromatography resin for IgG according to claim 20, wherein the the pair of constituent moieties are:

a)   ;

b)   .

22. An affinity chromatography resin for IgG according to either claim 20 or claim 21, wherein

the constituent moieties are linked together and to the sold support by a chemical linking group, which is of the formula:

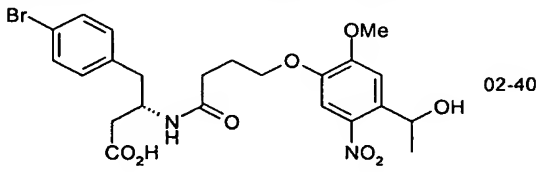


wherein X is selected from O or CH_2;

n is 0, 1 or 2; and

m is 2, 3 or 4.

23. An affinity chromatography resin for IgG according to any one of claims 20 to 22, wherein the affinity ligand is selected from: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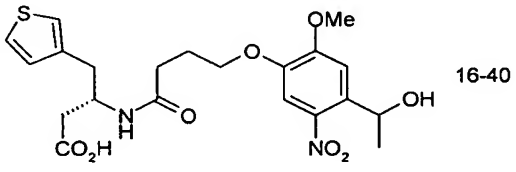
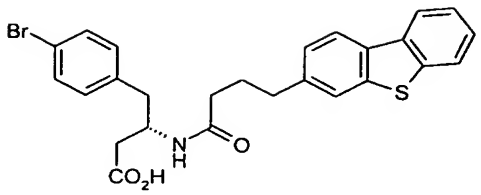
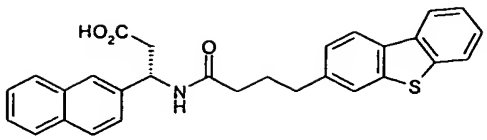

16-40



02-69



18-69

24. The use of the affinity chromatography resin according to any one of claims 20 to 23 for the purification of IgG.

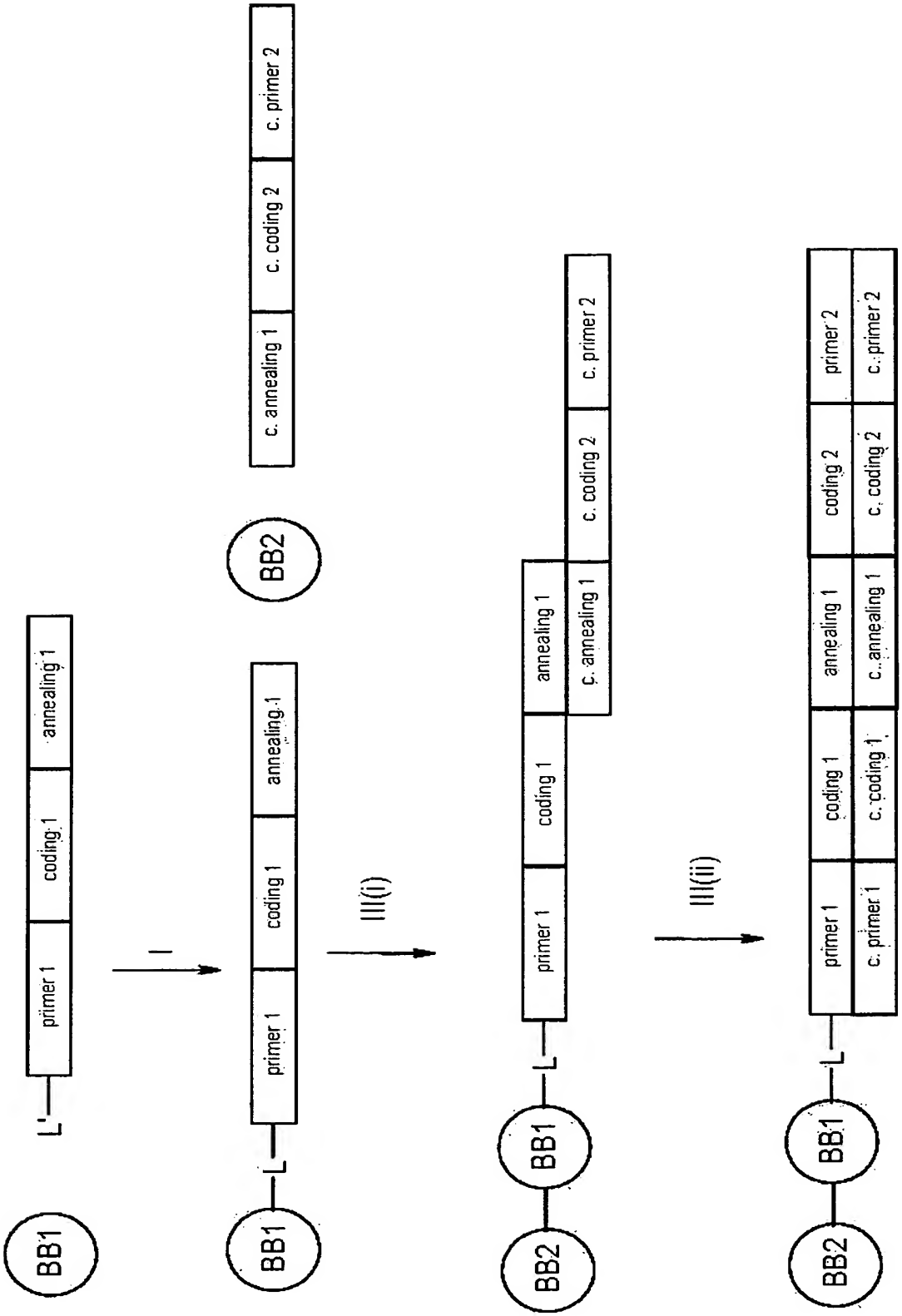

BB1
L'
primer 1
coding 1
annealing 1
I
BB1
L
primer 1
coding 1
annealing 1
BB2
c. annealing 1
c. coding 2
c. primer 2
III(i)
BB1
L
primer 1
coding 1
annealing 1
c. annealing 1
c. coding 2
c. primer 2
BB2
III(ii)
BB1
L
primer 1
coding 1
annealing 1
coding 2
primer 2
c. primer 1
c. coding 1
c. annealing 1
c. coding 2
c. primer 2
BB2

Fig. 1

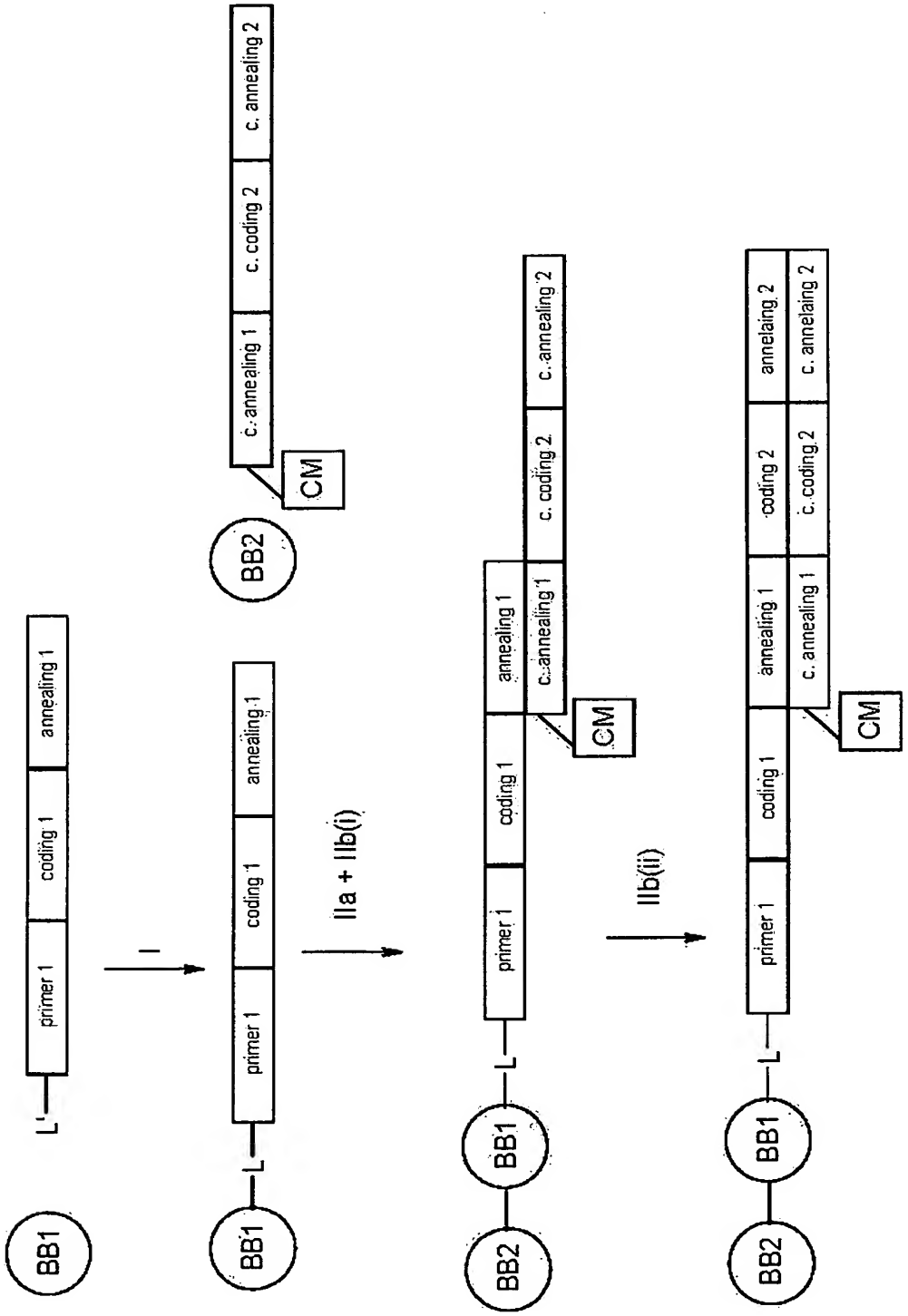

BB1
L'
primer 1
coding 1
annealing 1
I
BB1
L
primer 1
coding 1
annealing 1
IIa + IIb(i)
BB2
c. annealing 1
c. coding 2
c. annealing 2
CM
BB2
BB1
L
primer 1
coding 1
annealing 1
c. annealing 1
c. coding 2
c. annealing 2
CM
IIb(ii)
BB2
BB1
L
primer 1
coding 1
annealing 1
c. annealing 1
coding 2
c. coding 2
annelaing 2
c. annelaing 2
CM

Fig. 2i

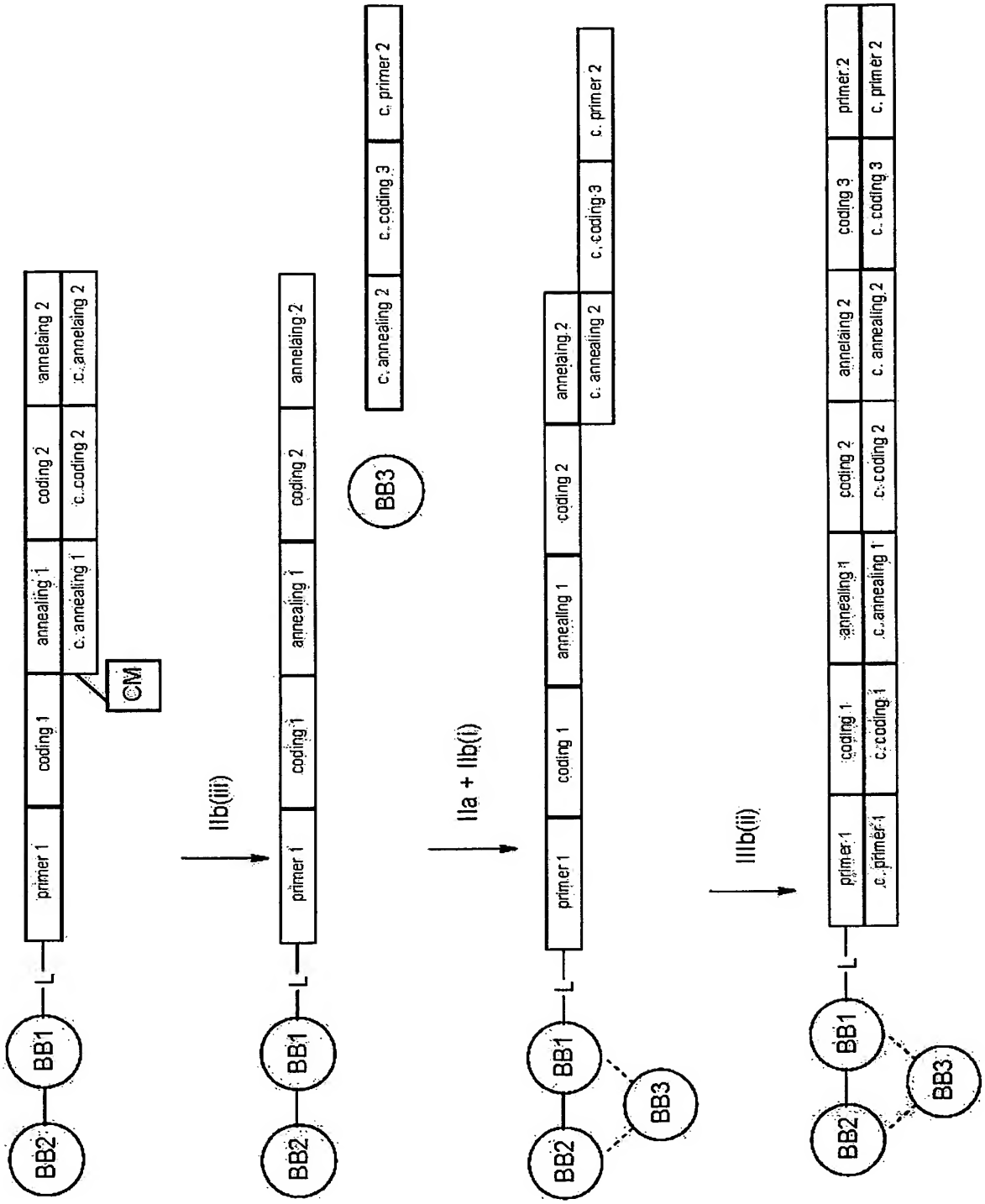

primer 1
coding 1
annealing 1
coding 2
annelaing 2
c. annealing 1
c. coding 2
c. annelaing 2
CM
BB1
BB2
IIb(iii)
primer 1
coding 1
annealing 1
coding 2
annelaing 2
BB1
BB2
BB3
c. annealing 2
c. coding 3
c. primer 2
IIa + IIb(i)
primer 1
coding 1
annealing 1
coding 2
annelaing 2
c. annealing 2
c. coding 3
c. primer 2
BB1
BB2
BB3
IIIb(ii)
primer 1
coding 1
annealing 1
coding 2
annelaing 2
coding 3
primer 2
c. primer 1
c. coding 1
c. annealing 1
c. coding 2
c. annealing 2
c. coding 3
c. primer 2
BB1
BB2
BB3

Fig. 2ii

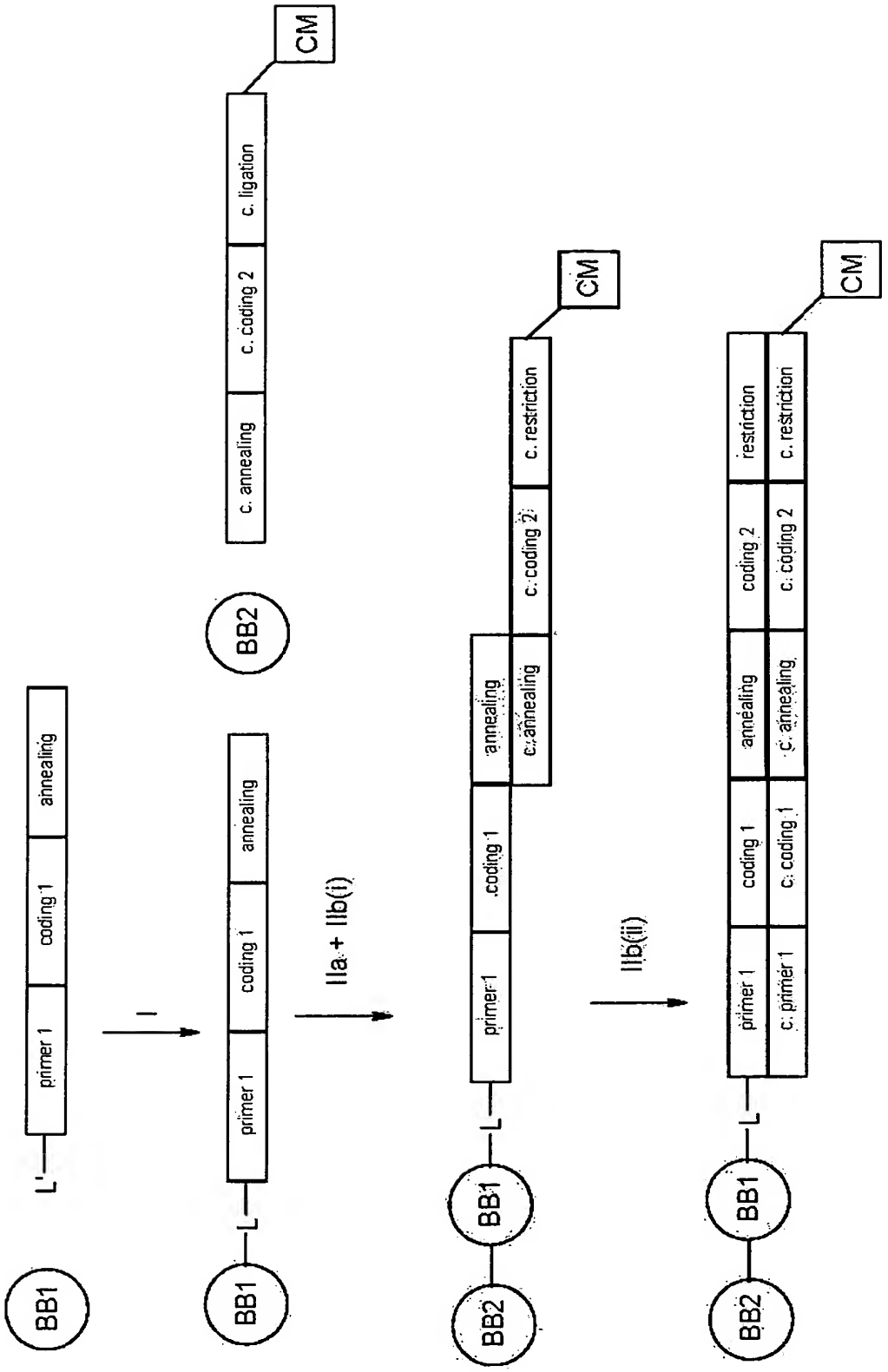

BB1
L'
primer 1
coding 1
annealing
I
BB1
L
primer 1
coding 1
annealing
IIa + IIb(i)
BB2
c. annealing
c. coding 2
c. ligation
CM
BB1
L
primer 1
coding 1
annealing
c. annealing
c. coding 2
c. restriction
CM
BB2
IIb(ii)
BB1
L
primer 1
coding 1
annealing
coding 2
restriction
c. primer 1
c. coding 1
c. annealing
c. coding 2
c. restriction
CM
BB2

Fig. 3i

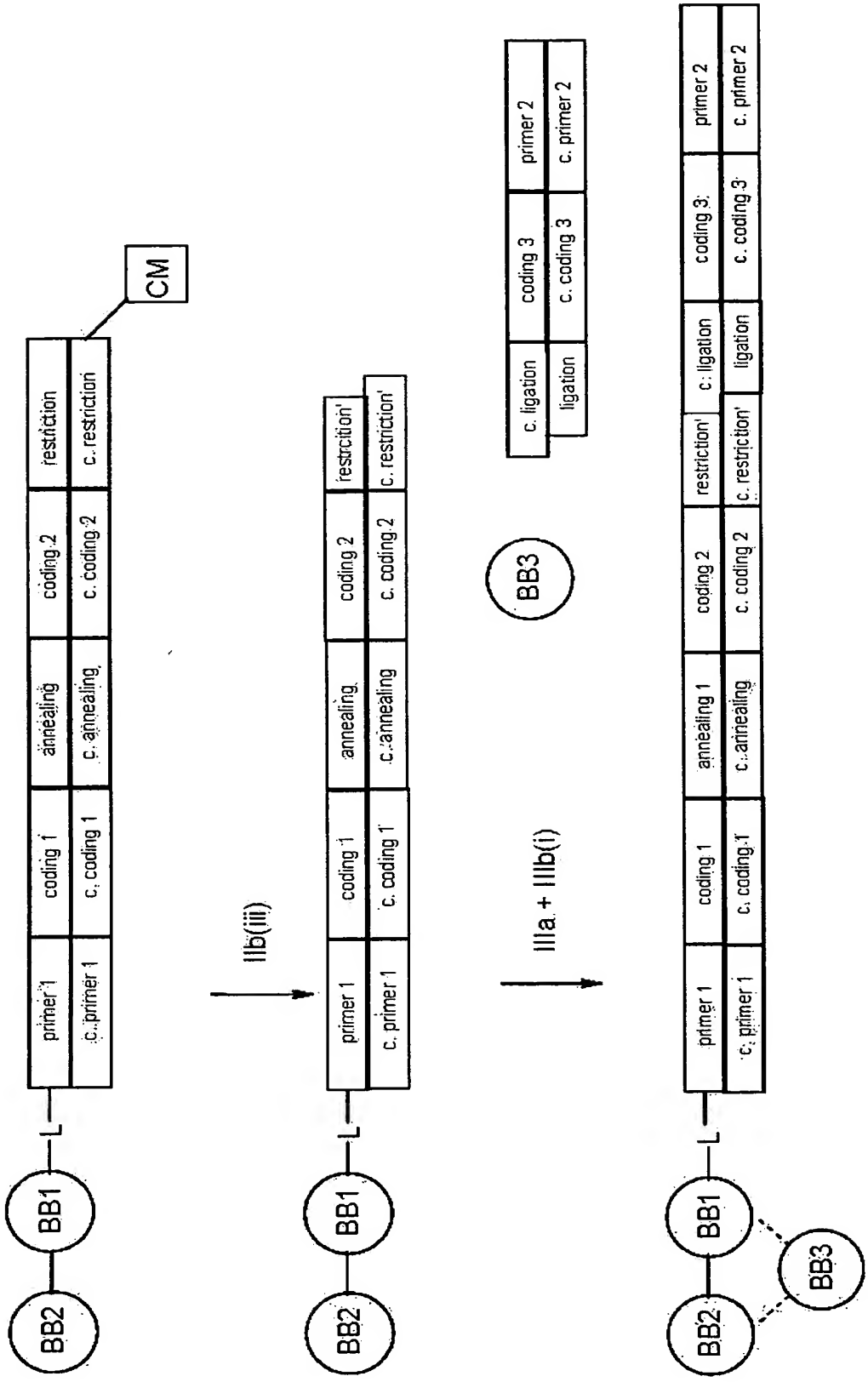

BB1
BB2
L
primer 1
c. primer 1
coding 1
c. coding 1
annealing
c. annealing
coding 2
c. coding 2
restriction
c. restriction
CM
IIb(iii)
restriction'
c. restriction'
IIIa + IIIb(i)
BB3
c. ligation
ligation
coding 3
c. coding 3
primer 2
c. primer 2
restriction'
c. restriction'
c. ligation
ligation
coding 3
c. coding 3
primer 2
c. primer 2

Fig. 3ii

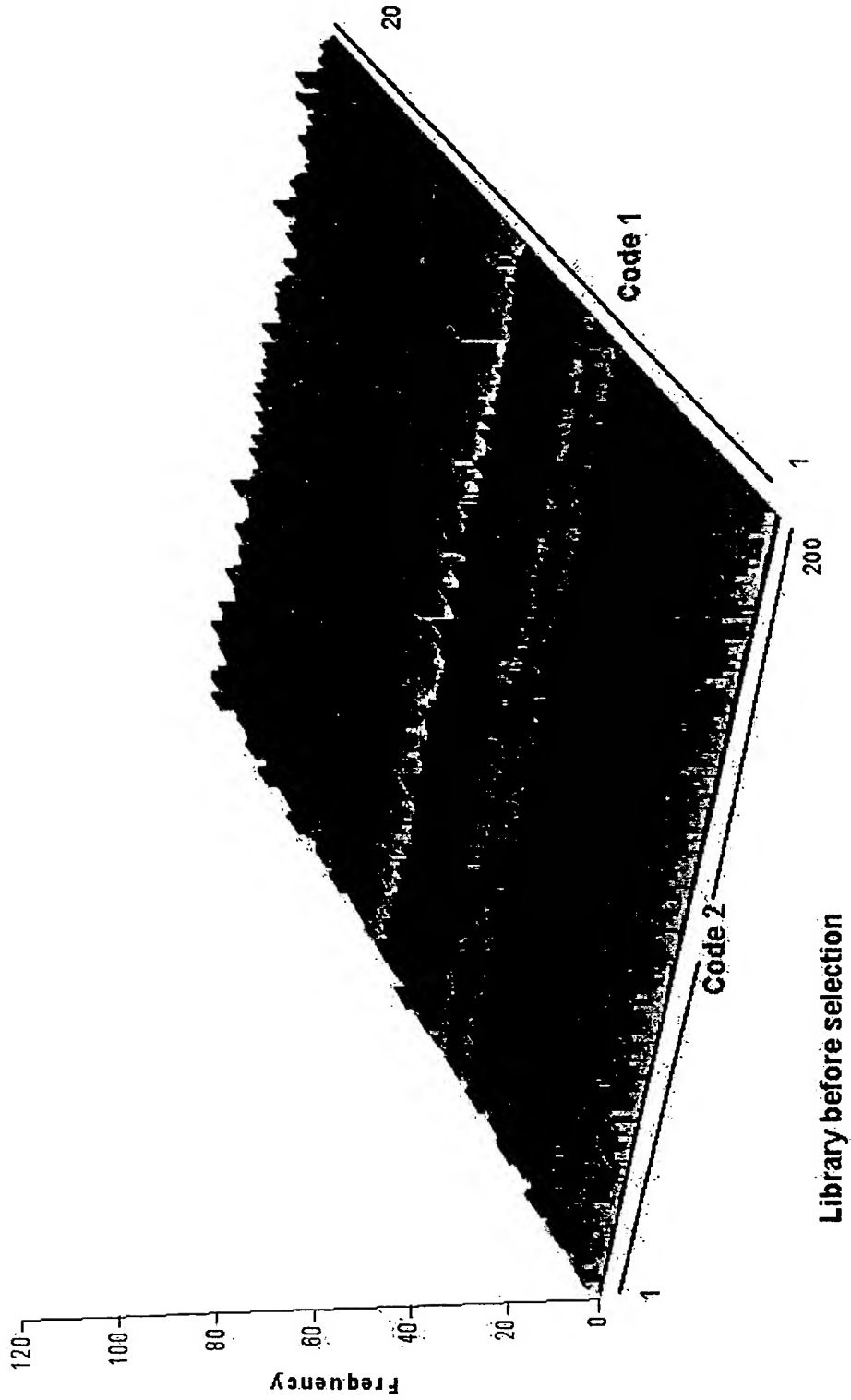

Frequency
120
100
80
60
40
20
0
1
Code 2
200
1
Code 1
20
Library before selection

Fig. 4a

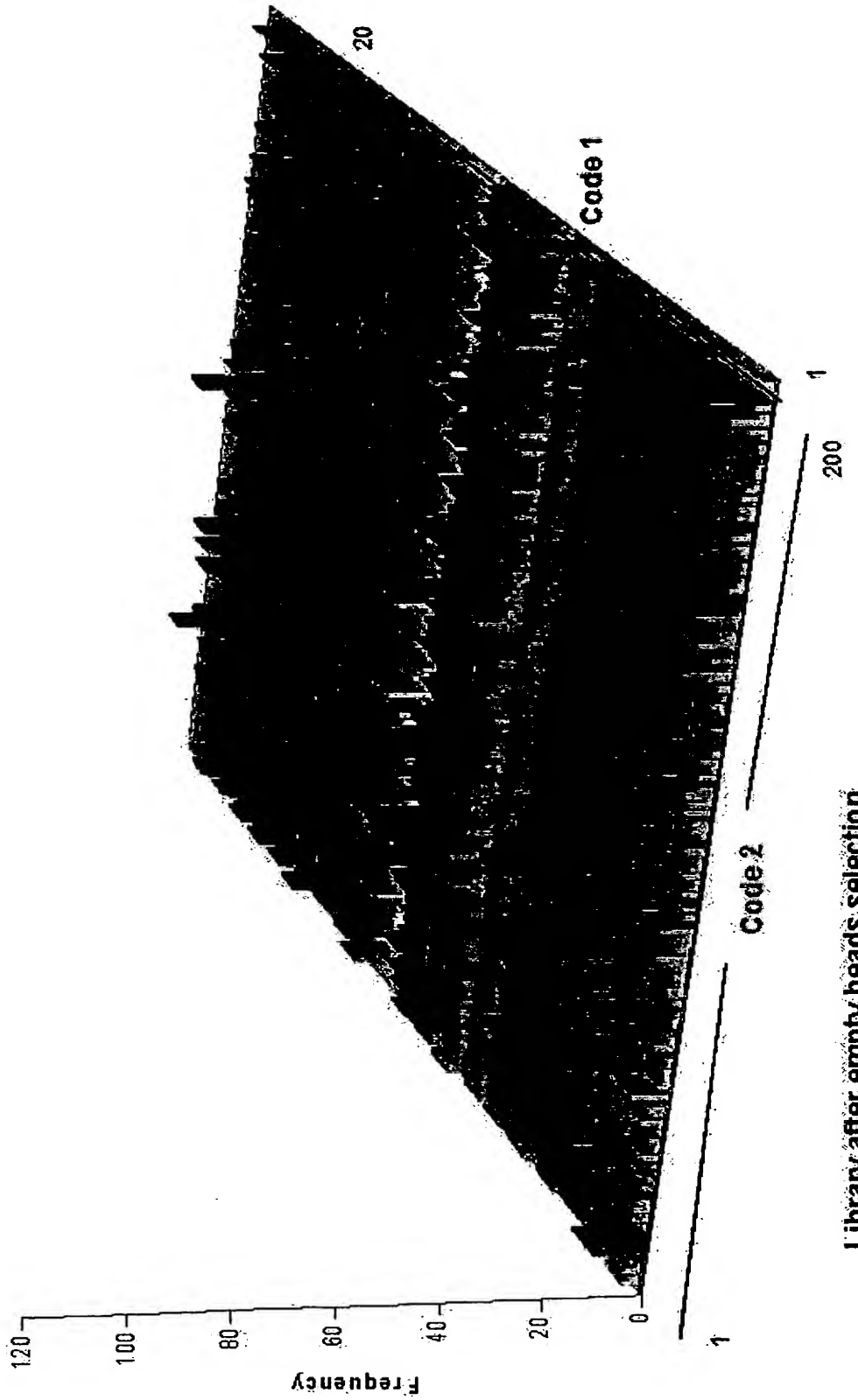

Library after empty beads selection
Code 1
Code 2
Frequency
120
100
80
60
40
20
0
1
200
1
20

Fig. 4b

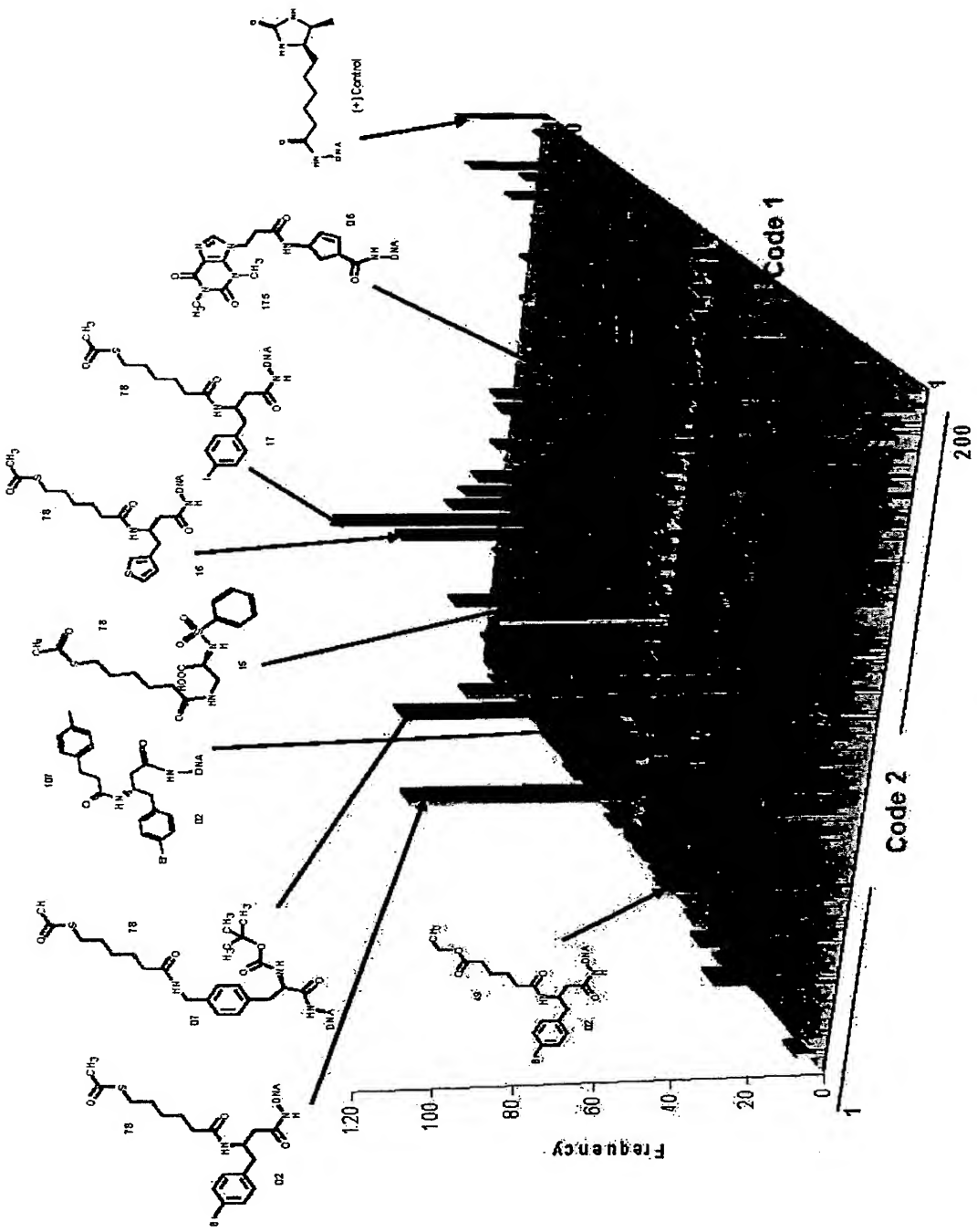

(+) Control
Code 1
Code 2
Frequency
120
100
80
60
40
20
0
1
200
Library after Streptavidin beads selection

Fig. 4c

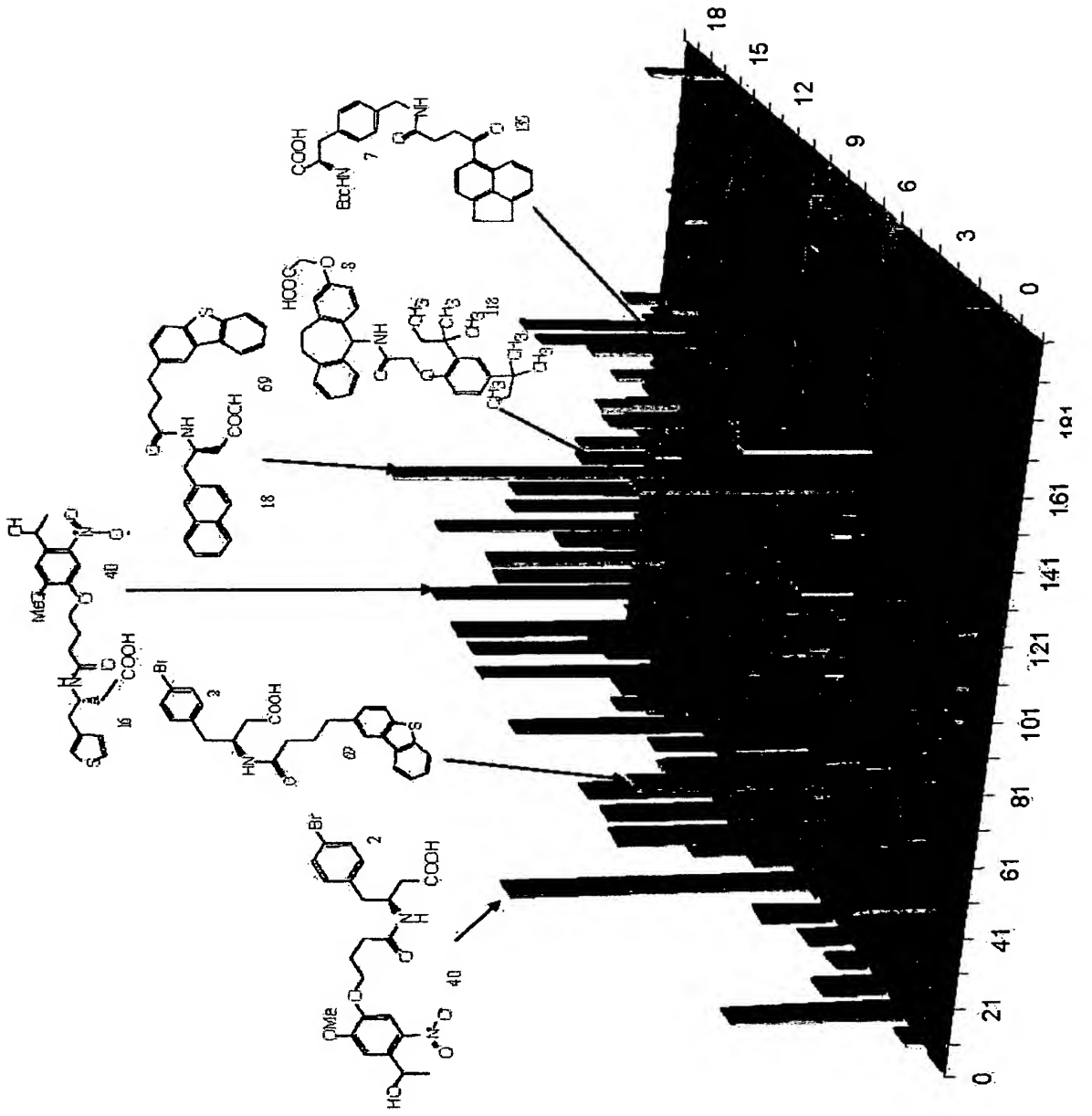


Fig. 5

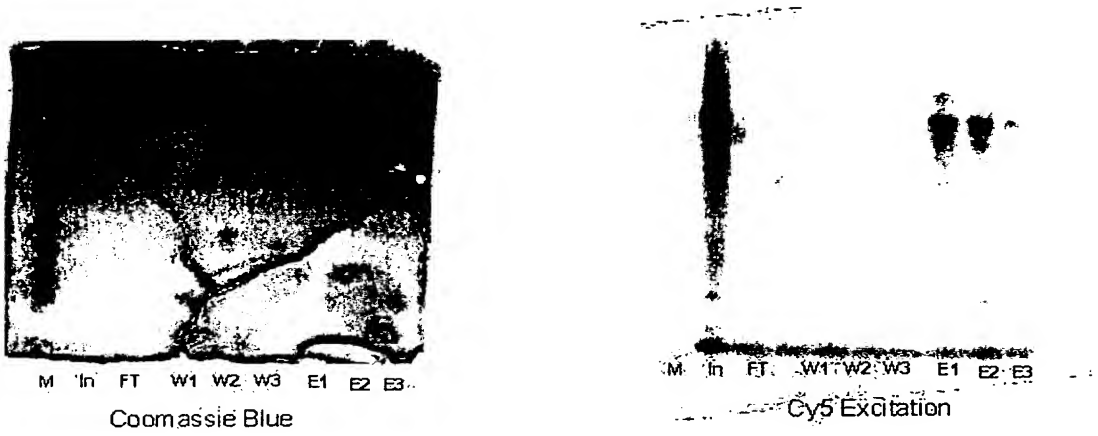

M In FT W1 W2 W3 E1 E2 E3
Coomassie Blue
M In FT W1 W2 W3 E1 E2 E3
Cy5 Excitation

Fig. 6a

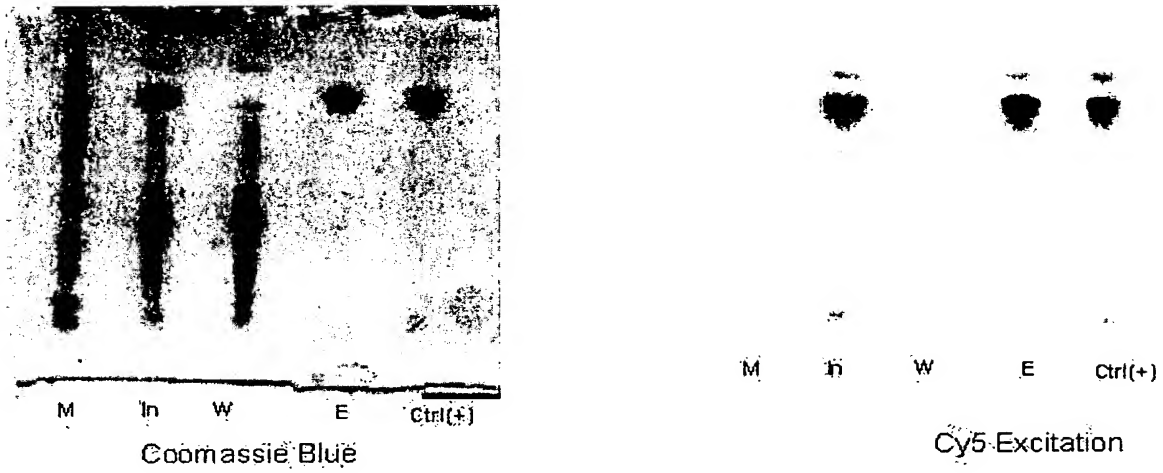

M In W E Ctrl(+)
Coomassie Blue
M In W E Ctrl(+)
Cy5 Excitation

Fig. 6b

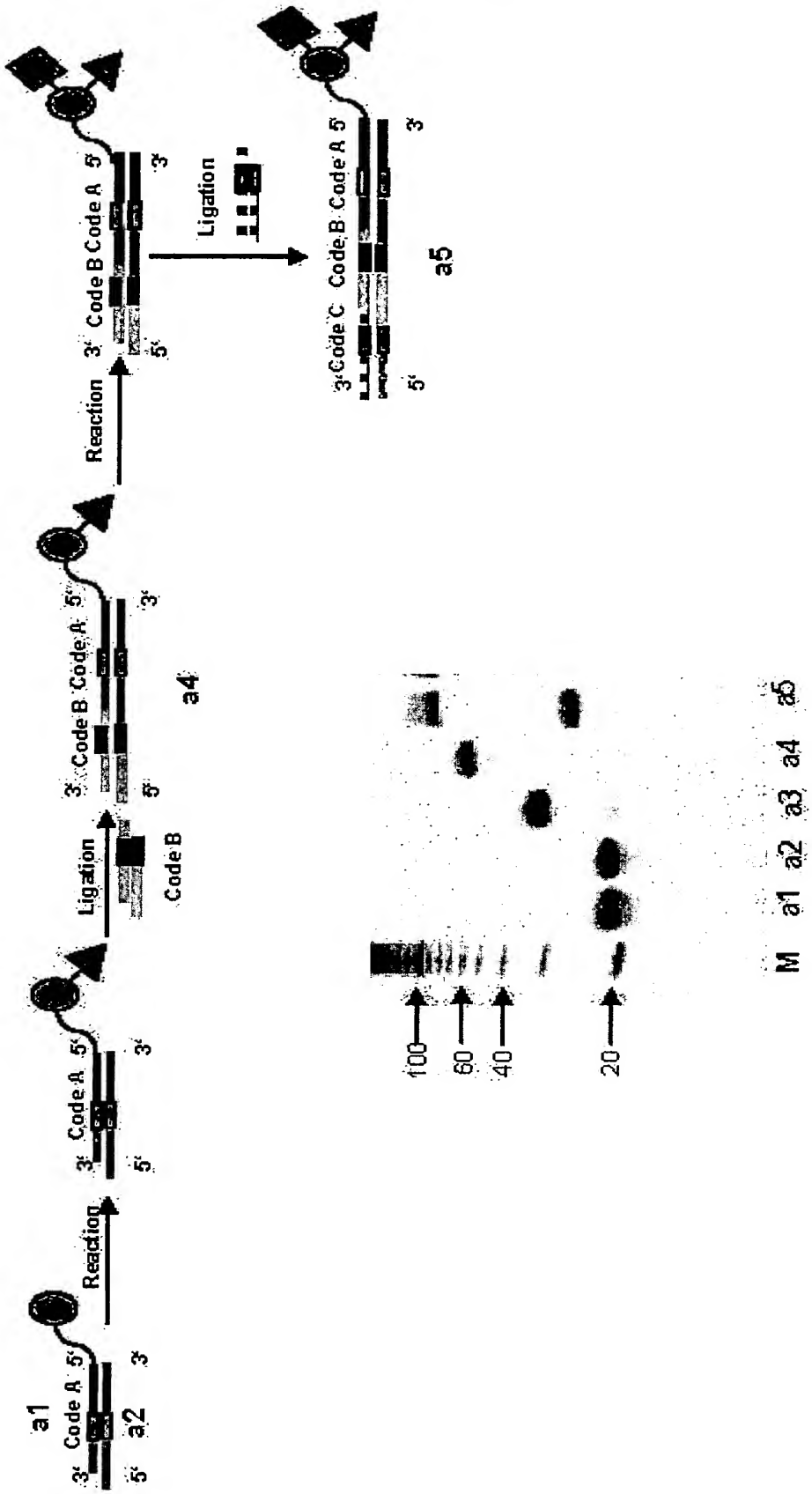
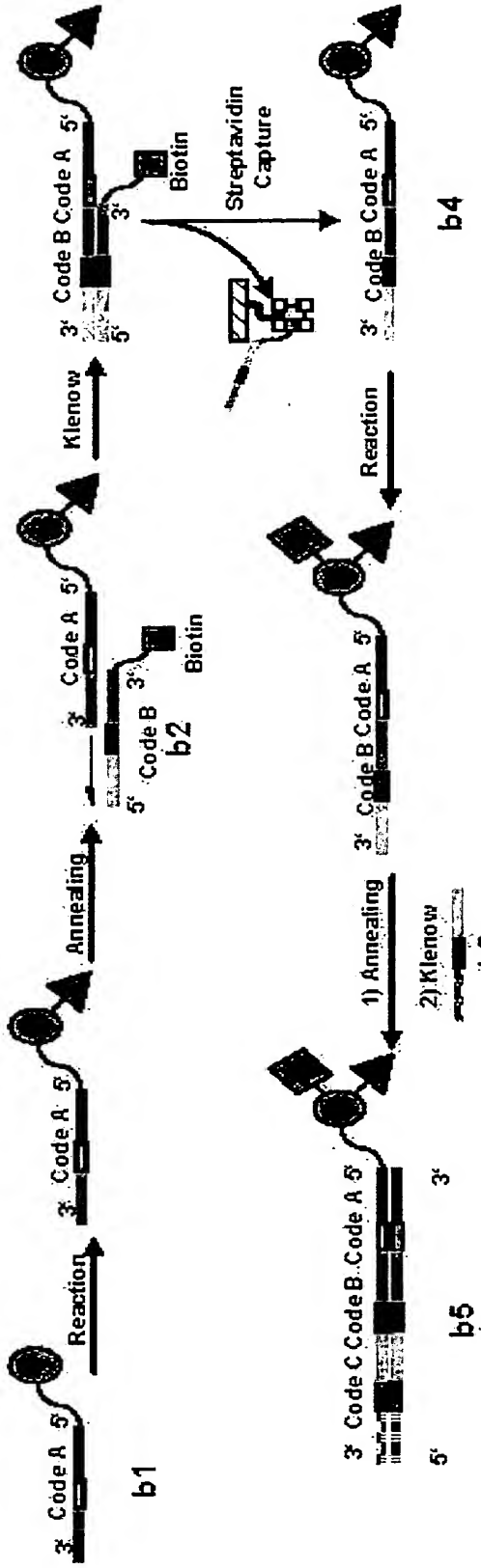

a1
Code A
a2
Reaction
Code A
Ligation
Code B
a4
Code B
Code A
Reaction
Code B
Code A
Ligation
Code C
Code B
Code A
a5
100
60
40
20
M
a1
a2
a3
a4
a5

Fig. 7a


Code A
Reaction
Code A
Annealing
Code B
Biotin
b2
Klenow
Code B Code A
Biotin
Streptavidin
Capture
b4
Reaction
Code B Code A
Code A
1) Annealing
2) Klenow
b3
Code C Code B Code A
b5
b1

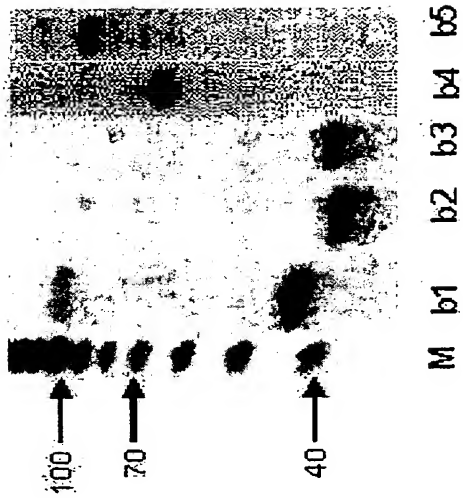
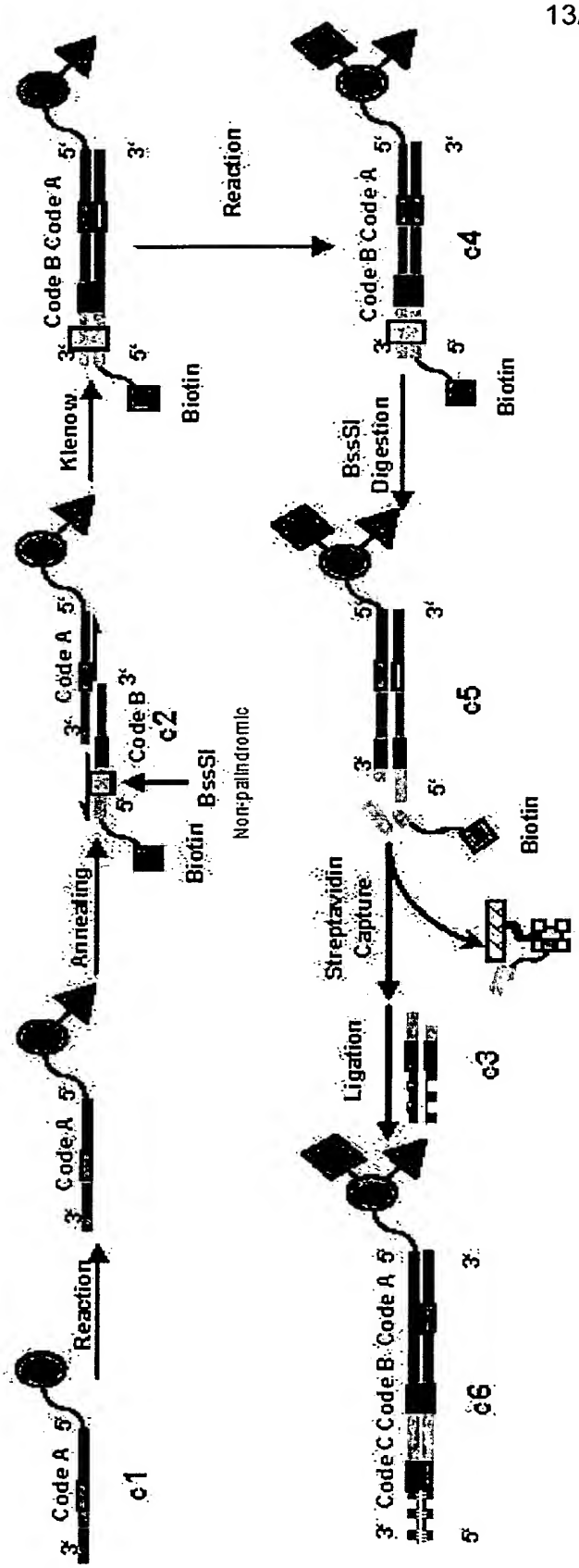

100
70
40
M b1 b2 b3 b4 b5

Fig. 7b


Code A
Code B
Code C
Reaction
Annealing
Klenow
Biotin
BssSI
Non-palindromic
c1
c2
c3
c4
c5
c6
Streptavidin Capture
Ligation
BssSI Digestion

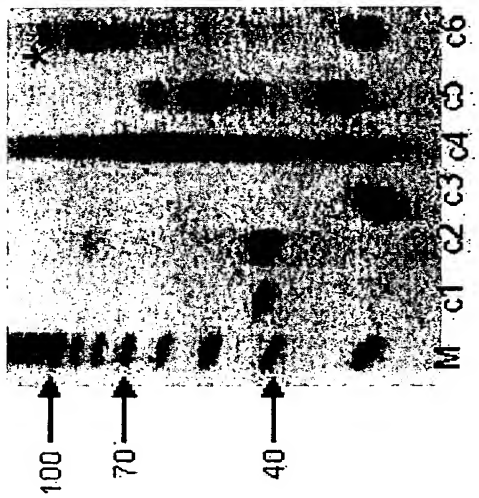

M
c1
c2
c3
c4
c5
c6
100
70
40

Fig. 7c

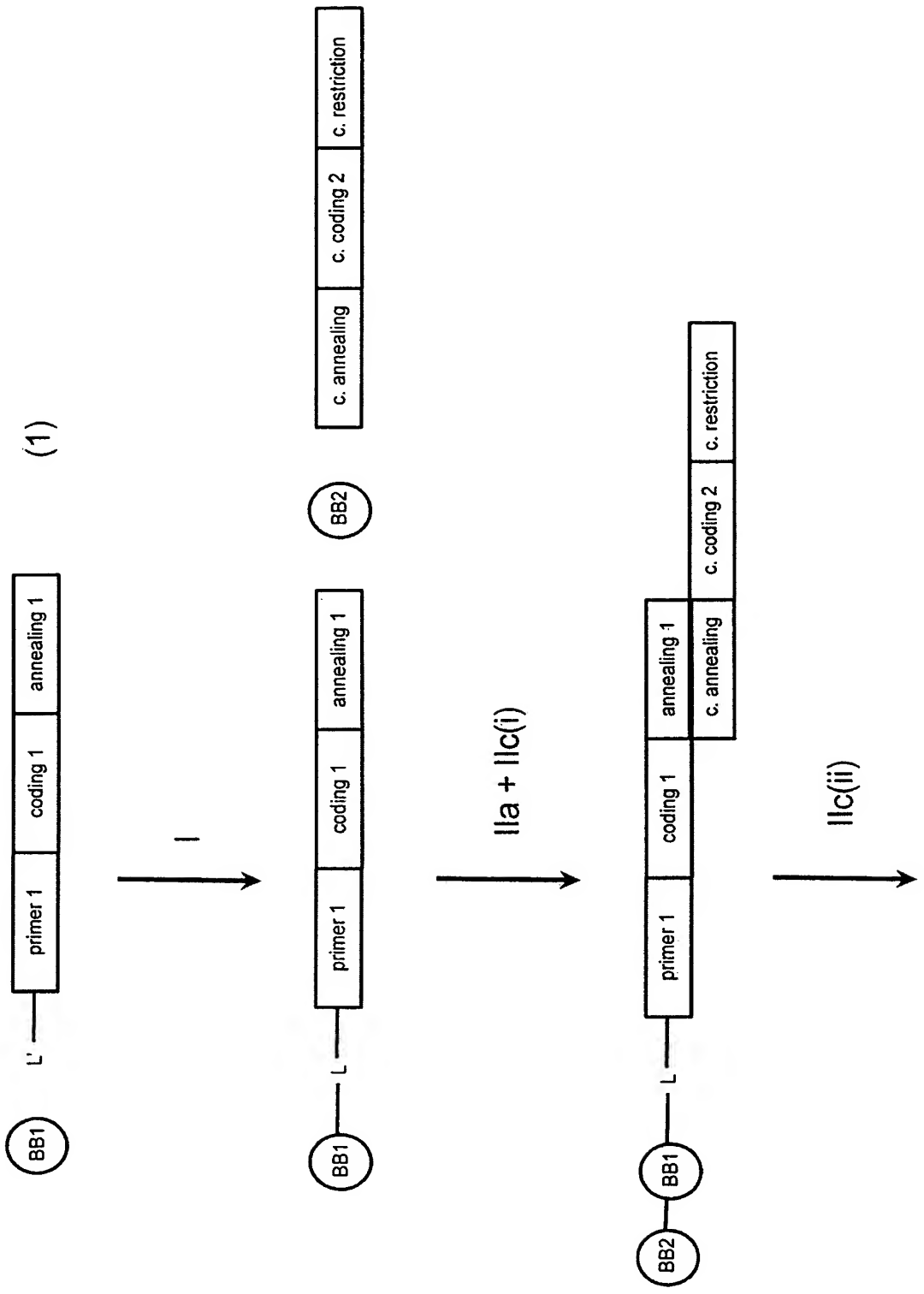

(1)
primer 1
coding 1
annealing 1
L'
BB1
I
primer 1
coding 1
annealing 1
L
BB1
c. annealing
c. coding 2
c. restriction
BB2
IIa + IIc(i)
primer 1
coding 1
annealing 1
c. annealing
c. coding 2
c. restriction
L
BB1
BB2
IIc(ii)

Fig. 8ai

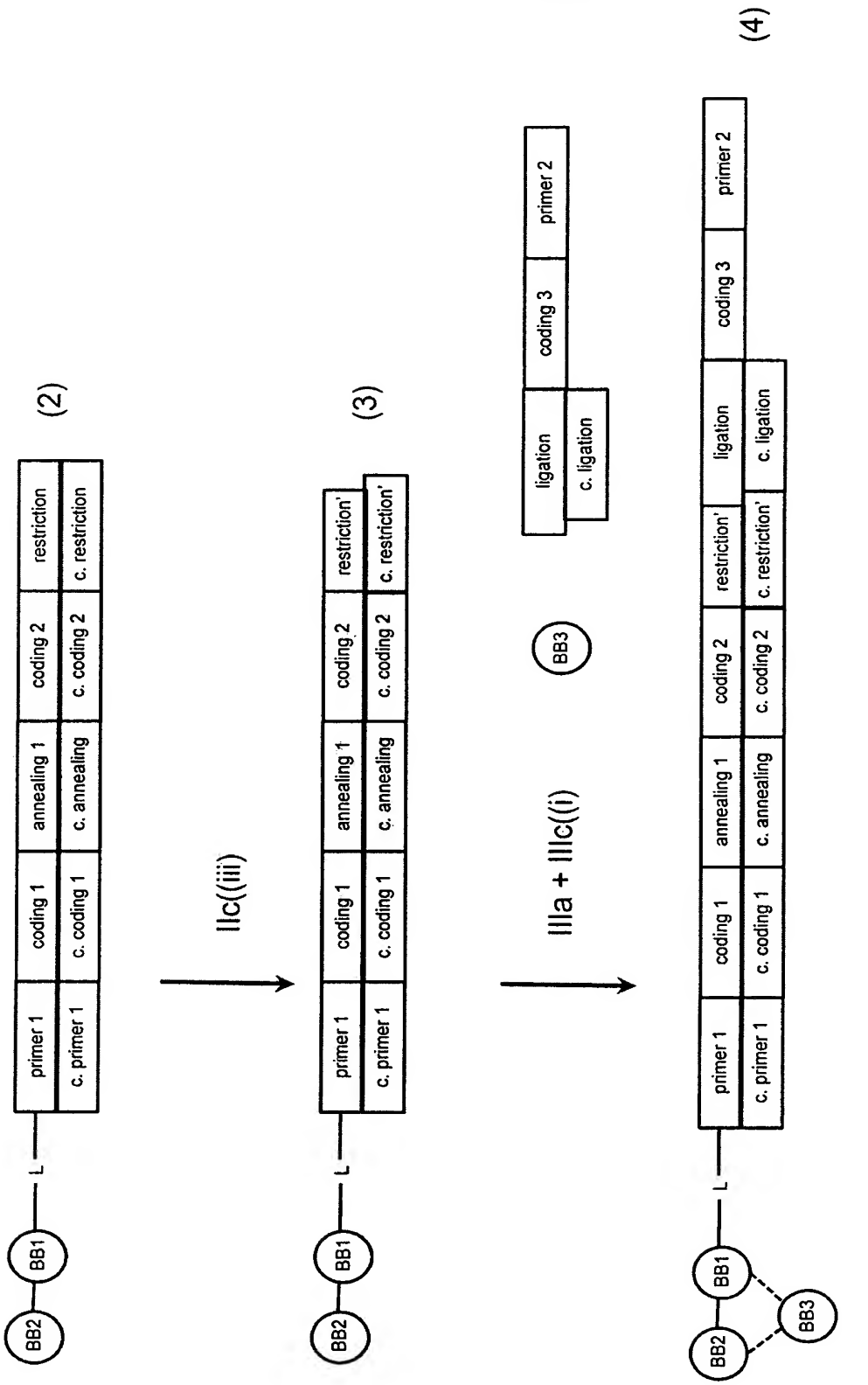

(2)
primer 1
coding 1
annealing 1
coding 2
restriction
c. primer 1
c. coding 1
c. annealing
c. coding 2
c. restriction
L
BB1
BB2
IIc((iii))
(3)
primer 1
coding 1
annealing 1
coding 2
restriction'
c. primer 1
c. coding 1
c. annealing
c. coding 2
c. restriction'
L
BB1
BB2
IIIa + IIIc((i))
BB3
ligation
coding 3
primer 2
c. ligation
(4)
primer 1
coding 1
annealing 1
coding 2
restriction'
ligation
coding 3
primer 2
c. primer 1
c. coding 1
c. annealing
c. coding 2
c. restriction'
c. ligation
L
BB1
BB2
BB3

Fig. 8aii

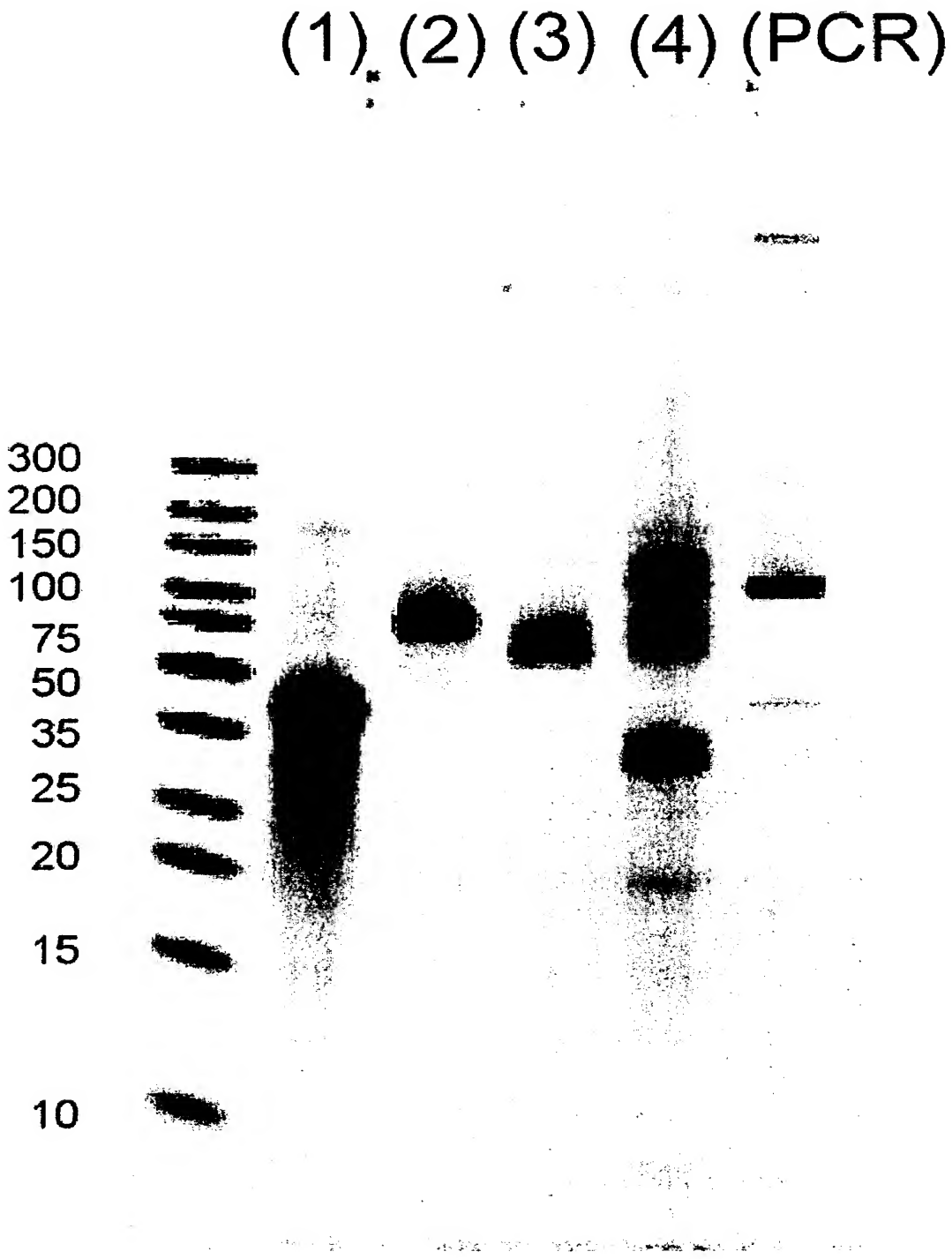


Fig. 8b